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CHEMICAL RESPONSES BY MARINE ORGANISMS TO STRESS. PHASE I

Harry Perry Jeffries

Rhode Island University
Kingston, Rhode Island

June 1967

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GRADUATE SCHOOL OF OCEANOGRAPHY
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PHASE I

Harry Perry Jeffries

June 1967

TECHNICAL REPORT NO. 1

October, 1965-June, 1967

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FWPCA

Approved for Distribution John A. Knauss

John A. Knauss
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SUMMARY

The long-term goal is to predict the consequences of environmental stress on estuarine biota. The homeostatic responses of fatty acids and free amino acids are sensitive indicators of environmental changes. Stress may take the form of seasonal temperature variation in the annual cycle of plankton production, or stress may result from artificial complications such as pollution.

Fatty acid levels in the plankton of the Narragansett Bay area (Rhode Island) are associated with seasonal replacement of species populations. During vernal warming, when the winter forms are replaced by warm-water species, the concentration of palmitic acid in the phytoplankton-microzooplankton shoots upward while palmitoleic drops an equal amount (about 25% of the total fatty acids). In the zooplankton, the weight ratio of palmitic to palmitoleic acids reflects the condition of the community. When the ratio decreases, the zooplankton is plentiful; an increasing ratio signifies environmental stress, reduced production and impending change of community organization.

Fatty acid composition of the plankton can be pictured as a three-dimensional surface to show relative stability of offshore and estuarine communities. The surface denotes biochemical integrity existing at the community level. Abnormal conditions accruing in the environment should be revealed by changes in the shape of the surface.

Free amino acid distributions in the plankton also reflect stability and the nature of the specific habitat. Blocks of communality within matrices of correlation coefficients are fingerprints of the environment's

ability to produce under varying conditions. These fingerprints could be used as sensitive indicators of adverse conditions.

Now that we know the important relationships between metabolic constituents and community biology, the minimum stress intensities and corresponding physiological tolerances are being measured in species populations:

(1) Incinerator residues have no effect on the fatty acid composition of the winter flounder, but the experiment must be repeated.

(2) Nearby populations of the hard clam have persistent differences in fatty acid composition. A genetic basis is apparent, in addition to induction by the specific environment.

(3) Serum protein patterns of a winter flounder population are associated with age, sex, maturation and disease. Individual variation occasionally obscures species specificity.

(4) Free amino acid concentrations in the small, ecologically important bivalve *Gemma gemma* are proportional to salinity. The relationship is influenced by temperature, sediment and feeding.

(5) There is an evolutionary adaptation of free amino acid levels in the planktonic copepod species representing open ocean, coastal and estuarine environments.

PREFACE

Specific Aims

This investigation explores the relationships between ecological stress and homeostatic response in marine organisms. A change in the environment causes a pattern of chemical change within an organism. When the patterns of response are understood, we should be able to predict changes in abundance that result from natural and artificial factors.

Predictions of this sort are relevant to water quality control. Pollutants can be regarded as stressors and the patterns of response are characteristic. Since the responses are homeostatic, they foretell external conditions which, if not abated, could lead to death and reorganization of the community.

Three specific aims were proposed:

Aim 1: To identify metabolic constituents that change in relation to distribution and abundance of marine communities.

Aim 2: To measure chemical specificity of populations in terms of genetic history and environmental stress.

Aim 3: To find the minimal stress intensities and corresponding physiological maximum tolerances within which normal body composition and function are maintained.

These aims, embodying the concepts of stress, acclimation, adaptation and evolution are broad,but they can be approached collectively with the idea that the responses relevant to a practical goal can be screened rather quickly and the most sensitive approach selected for continued observation. We have found that the patterns of free amino acids, fatty acids and the major lipid classes comprise a sensitive approach to a broad problem.

Starting with major natural marine communities, we now know the stress intensities and corresponding maximum tolerances within which the normal body composition of fatty acids and free amino acids are maintained. The responses of these components, varied as they are, are sensitive indicators of metabolic sequences in the homeostatic process. Observations continue on specific patterns both in nature and in the laboratory so as to predict, for example, just what effect a given pollutant might have on an estuarine species and its community.

Secondary Aims

Three secondary interests are related to the long-term goal of predictability:

1. Determine shifts in fatty acid and amino acid composition during the process of lipid and nitrogen transfer up the food chain.
2. Determine if the dominant organisms in major marine communities have unique fatty acid and amino acid pools.
3. Relate the above to functional morphology of individuals and the energetics of communities.

With this perspective, the specific aims are relevant to water quality control, and they assume a level of general significance.

> *The maintenance of dynamic equilibrium or steady state constitutes homeostasis, the ability to survive in a varying environment -- the central physiological characteristic of living things.* (Prosser 1964).

I. Introduction.

This report covers the first two years of an investigation planned for a five-year period. The specific aims are to be accomplished in three successive phases: (1) observation of chemical responses in natural plankton communities; (2) experimental determination of homeostatic behavior under defined conditions and; (3) resynthesis of the observational and experimental phases at the community level to show the nature of compensatory responses.

A. Perspectives on the specific aims.

The observational phase has been completed. This report considers patterns of seasonal change in the composition of plankton communities. The correspondence between chemical composition and species composition of the community is evidently very close. Furthermore, the patterns of chemical composition reveal a continuum of change indicative of seasonal variations in environmental stress.

Progress has also been made on the experimental phase. Three graduate students are studying the responses of important estuarine species to known conditions in nature and in the laboratory. Mr. Gerald Pesch completed a Master's thesis on the natural variations of fish plasma proteins. Mr. Ross Wilcox is studying the osmotic significance of free amino acids in Gemma gemma. Miss Laima Alzara's thesis deals with the relationships between osmotic stress and internal tolerances of marine and estuarine copepods. Mr. Pesch and Mr. Wilcox will continue in the doctoral program, developing the ideas brought out in their master's theses.

To maintain perspective on the relevance of this investigation, informal cooperation has been fostered with the National Marine

Water Quality Laboratory. Cooperation ranges all the way from exchanging information to chairing graduate committees. One member of the Water Quality Laboratory is now enrolled part-time as a Ph.D. candidate under the direction of this principal investigator. His techniques have been used in this study, and we look forward to an even closer association.

B. The major responses.

We have selected for continued observation the responses of free amino acids, fatty acids and the major lipid classes to ecological stress. Collectively, the quantitative variations of these biochemical intermediates and end products reveal the tolerances of homeostatic mechanisms. More than 60 compounds have been identified. Simple relationships exist between the internal milieu and the external environment, but the multivariate nature of these mechanisms is becoming increasingly clear. Their patterns have dimensions, and the dimensions signify the fitness of the environment.

II. Plankton Sampling and Analytical Procedure.

A. Plankton sampling.

Samples were taken in Narragansett Bay, Rhode Island and Rhode Island Sound from October 1963 to January 1965 and from October 1965 to September 1966. During the first period, the stations were: A, 12 miles within the West Passage of Narragansett Bay, and B, in Rhode Island Sound near the mouth of the Bay. Two nets were used at each station: a number 20 mesh net, 0.33 m dia, towed at the surface, and a number 2 mesh net, 0.5 m in dia, towed obliquely through the water column.

The small, fine net (No. 20) caught diatoms and immature stages of the zooplankton, an important link in the food chain between the small primary producers (<10μ) and the macrozooplankton (Conover 1964). This size group is considered a community here, and it is called the phytoplankton-microzooplankton.

The large, coarse net (No. 2) retained late copepodite stages, immature fish, crab and shrimp larvae and chaetognaths. This size group is referred to as the zooplankton.

During the second observation period (1965-66), the Bay station was moved downbay, 5 miles from the mouth. The Sound station was moved seven miles offshore from its former position near the entrance to the Bay.

During the first sampling period, most of the water was drained from the plankton. The sample was kept in an ice chest for about an hour, and then the extractions were made. The long-chain fatty acids were oxidized, so the procedure was modified in the 1965-66 program. The samples were immediately drained of all water using wide mouth bottles fitted with gauze screen. After filling the bottle and inverting, a small vent was opened in the bottle, and the plankton drained on the screen. Water was injected through the vent to wash all the plankton onto the screen. Then the bottle was sealed in a plastic bag and refrigerated at 0°C. The samples were immediately delivered by boat to the laboratory, weighed and freeze dried. This procedure prevented the oxidation of long-chain fatty acids (see Tables 2 and 3).

During the second period, the zooplankton was sampled quantitatively using an enlarged Clarke-Bumpus sampler fitted with a

number 2 mesh net. If desired, the results, whether copepod numbers, free amino acids in the plankton or lipid content, can be expressed in terms of cubic meters of sea water or square meters of sea surface.

B. The analysis of fatty acids, free amino acids and the major lipid classes in small quantities of marine organisms.

A routine analysis requires 2-3 g (wet weight) of sample. After freeze drying, the sample is stored in a vacuum dessicator at -20C and then extracted with a chloroform:methanol:water solvent. The free amino acids are dissolved in the epiphase and the total lipids in the hypophase, as shown in the accompanying flow chart. The procedure is designed to permit a complete analysis on one small sample.

FLOW SHEET

AMINO ACID AND LIPID ANALYSES
(Numbers in parentheses refer to notes at the end of the diagram)

SAMPLE

Plankton (1), muscle, hepatopancreas, blood, hemolymph (2): 2-3 g portions freeze dried in tared flasks, stored under N_2 or in a vacuum dessicator and held at -20C.

0.5 g dry weight

Extract with monophasic $CHCl_3$-CH_3OH-H_2O [1:2:0.8] Homogenize. Add $CHCl_3$ and water [2:2:1.8]. Biphasic. (3)

Filter into separatory funnel through glass fiber

Precipitate

Epiphase: H_2O soluble materials

Evaporate under vacuum @ 50C. Re-suspend in 10% isopropanol. Centrifuge.

Residue

Extract: free amino acids (5)

Determine total concentration of α amino N (6). Adjust concentration of extract to 45-90 µM/ml.

AMINO ACID ANALYSIS by ion-exchange chromatography (7)

Add internal standard (norleucine) and sucrose to sample; inject 0.2 ml.

Quantification: measure peak areas by triangulation; correct areas with respect to response of the internal standard; refer corrected areas to standard areas for each amino acid [0.2 µM]. Calculate µM.

Inter-facial floc (proteolipids)

Hypophase: most lipids (4)

TOTAL LIPID FRACTIONATION by thin layer chromatography

Determine chromatographic parameters for particular extract with "microplates" (8). Apply extract to 20 cm silicic acid basic plates (9), develop and visualize with I_2, UV or H_2SO_4:$K_2Cr_2O_7$ spray.

Quantification:
preparative plates -- elute and determine amounts gravimetrically (9) or colorometrically (10); thin plates -- measure spot areas planimetrically and refer to standards (11).

FATTY ACID ANALYSIS by gas chromatography (cont.)

Determine % water
Determine total N by micro-Kjeldahl. Refer analyses to dry weight or to N content.

FATTY ACID ANALYSIS by
Gas Chromatography

Evaporate hypophase to dryness at 40C under vacuum and N_2. Dissolve residue in pet ether and transfer to reflux flask. Evaporate solvent with N_2.

Cod liver oil "standard" (12)

Saponification: Reflux with KOH-CH_3OH. Dilute soaps with distilled H_2O and partition with pet ether to remove nonsaponifiables (13).

Saponifiable Material

Acidify with H_2SO_4 and extract fatty acids with pet ether. Evaporate under N_2.

Methylation (14)

Add BF_3:CH_3OH reagent to sample (dry) in reflux flask. Reflux for 10 min at 100C. Wash into separatory funnel with H_2O and add pet ether. Concentrate ether phase under N_2.

Conditions for Chromatography (15)

a) temperatures (C)

injection port	220
detector	220
column	190

b) Columns: Diethylene glycol succinate, 15% on Chrom W 80/100, acid washed, DMC treated. Dual columns, 6' x 1/8".

c) Gas flow (ml/min):He carrier, 45; air, 600; hydrogen, 45.

d) Inject: 0.2-0.4 µl

Non-saponifiables

The lipids remaining in this fraction can be analyzed by thin layer chromatography when necessary to check the saponification procedure and the fractionation of total lipids.

Quantification

Measure retention times relative to methyl stearate. Identify peaks referring to retention data for standard fatty acids (16). Verify by hydrogenating and rechromatographing (18) or by fractionating the unsaturated fatty acids with nitromethane (19). Measure peak areas by height X width at half height (17). Calculate weight % or each fatty acid.

References and Notes -- Flow Sheet

(1) Preprocessing: nekton removed from plankton; fibrous tissues homogenized with water.

(2) For analysis of the free amino acids in blood, the sample can be deproteinized with 12% sulfosalicylic acid and the extract injected directly into the amino acid analyzer. (Oreskes, Cantor and Kupfer 1965).

(3) Bligh and Dyer (1959).

(4) Entenman (1961).

(5) This method has been compared with the procedure for free amino acid extraction given by Awapara (1948).

(6) Moore and Stein (1954).

(7) Technicon amino acid analyzer, model NC-1 (Ardsley, N.Y.), employing Chromobeads Type B for 18 hr analysis (high resolution) and Type C for 4 hr analysis (survey).

(8) Peifer (1962).

(9) Komarek, Jensen and Pickett (1964).

(10) Amenta (1964), Dittmer and Lester (1964), Kinsella (1966).

(11) Purdy and Truter (1962).

(12) Ackman and Burgher (1965).

(13) Burchfield and Storrs (1962) p. 494.

(14) Metcalf and Schmitz (1961).

(15) Perkin Elmer, Model 801, dual flame ionization detector.

(16) Ackman, Burgher and Jangaard (1963).

(17) Burchfield and Storrs (1962) p. 122.

(18) Ogg and Cooper (1949).

(19) Jangaard (1965).

III. Seasonal Responses of the Plankton to Thermal Stress and Associated Environmental Factors.

A. Ecological responses: succession of plankton populations.

Figure 1 shows the seasonal distribution of temperature, chlorophyll a and pigment ratios in Narragansett Bay and Rhode Island Sound. The winter minimum is 1-2C and the summer maximum reaches 20-22C. This range far exceeds the compensatory abilities of most planktonic organisms. A high level of production is nevertheless maintained, as the chlorophyll distribution in Figure 1 indicates, by a succession of species populations in the plankton. Each population is better adapted to the prevailing conditions than its forerunners.

The changeover from northern, cold water species to temperate and tropical species occurs in the spring and early summer. The pigment ratio D_{430}/D_{665} (Margalef 1963) falls to a minimum indicated by the heavy arrow in Figure 1. This low point preceeds succession from a community dominated by winter diatoms to a community dominated by warm-water flagellates. Figure 2, adapted from Pratt (1959), shows the diatom-flagellate succession in Narragansett Bay. Near the Bay mouth (lower graph) succession from periods 1 to 2 occurs during May. In the Bay (upper graph) the changeover is later, in late May and June.

Patterns of the zooplankton are shown by Figure 3. The percent composition of each major species is plotted against time in a three-dimensional graph. The winter-spring component, which emanates from Boreal waters, is replaced by three numerically important summer-fall species led by *Acartia tonsa*. During the

Figure 1. Distributions of temperature, chlorophyll *a* and the pigment ratio $D_{430m\mu}/D_{665m\mu}$ in Narragansett Bay and in Rhode Island Sound, near the mouth of the Bay. January 1964 to January 1965.

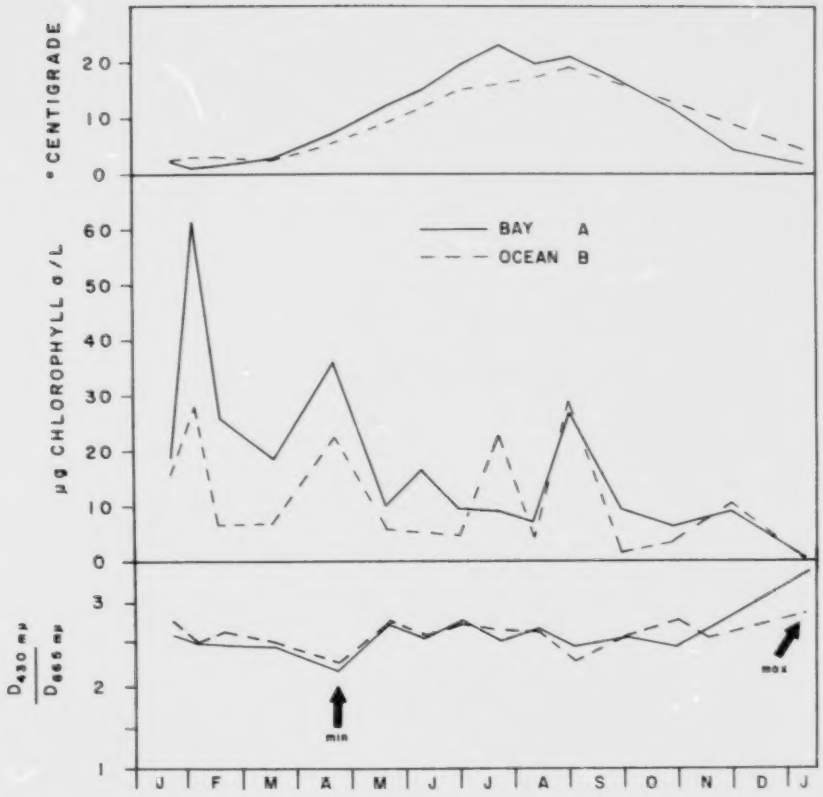
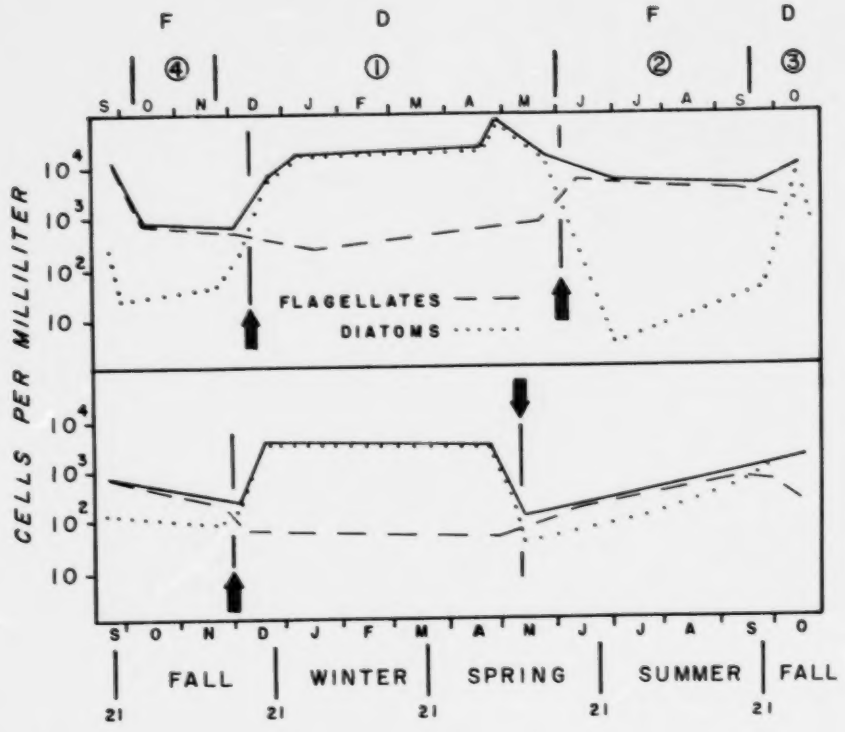

°CENTIGRADE
20
10
0
µg CHLOROPHYLL a/L
60
50
40
30
20
10
0
BAY A
OCEAN B
$\frac{D_{430\ m\mu}}{D_{665\ m\mu}}$
3
2
1
min
max
J F M A M J J A S O N D J

Figure 2. Schematic representation of summer and winter phytoplankton communities near the mouth of Narragansett Bay (lower) and 10 miles within the Bay (upper). Adapted from Pratt (1959).


F
D
F
D
④
①
②
③
S O N D J F M A M J J A S O
CELLS PER MILLILITER
10^4
10^3
10^2
10
FLAGELLATES
DIATOMS
FALL
WINTER
SPRING
SUMMER
FALL
21
21
21
21
21

periods of changeover -- in the spring and again in the fall -- temperature and associated environmental factors create conditions of increasing stress on the community. Internal responses should be apparent at these times as homeostatic mechanisms compensate for a progressively deteriorating environment. Further, the responses should disclose an imminent change of major consequence in the community, and since the responses occur before death verges on the limits of toleration they are predictors of natural phenomena.

These changes are not, however, revealed by simple proximate analyses on the plankton (Table 1). In the Bay, the amount of zooplankton or biomass/m^3 is high at the beginning of the year; it decreases in the spring and early summer and then increases to variable levels during the summer as trophic relationships become established (Martin, 1965). The Sound zooplankton increases in a regular manner during this period. Nitrogen content is approximately 10-11% throughout the year, and although the lipid content ranges far, the trend is obscure.

B. Chemical responses: fatty acid distributions.

The patterns of fatty acid composition in both the phytoplankton-microzooplankton and the zooplankton reveal a remarkable procession of events. Typical gas chromatograms of fatty acid methyl esters are shown in Figures 4 and 5. Many differences are clear to the eye.

The myriad changes occurring in the fatty acid composition can be portrayed with three-dimensional drawings. In Figures 6 and 7, time is the horizontal axis, percent concentration is the vertical axis, and the various fatty acids are arranged on the

Figure 3. Seasonal distribution of the dominant species in the winter-spring and summer-fall zooplankton communities in Narragansett Bay. Percent abundances of the total holoplanktonic species. January 1964 to January 1965.

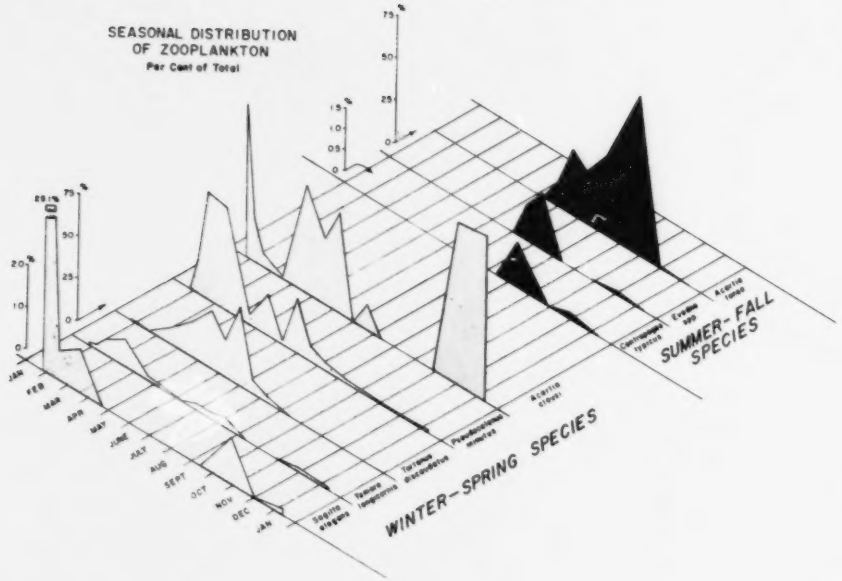

SEASONAL DISTRIBUTION
OF ZOOPLANKTON
Per Cent of Total
75
50
25
0
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
JAN
Sagitta elegans
Tomopteris longicornis
Tortanus discaudatus
Pseudocalanus minutus
Acartia clausi
WINTER–SPRING SPECIES
Centropages typicus
Evadne spp.
Acartia tonsa
SUMMER–FALL SPECIES

TABLE 1.

Dry weight, lipids and total nitrogen in zooplankton from Narragansett Bay and Rhode Island Sound (No. 2 mesh net, oblique tows). The values for lipids and nitrogen are percentages referred to dry weight of the sample. October, 1965 - September, 1966.

Date	Dry weight, mg/m^3		Percent lipid		Percent N	
	Bay	Sound	Bay	Sound	Bay	Sound
1965						
Oct. 6	19.1	8.7	11.9	7.1	8.6	6.5
Nov. 3	80.4	6.4	10.4	12.4	11.1	10.3
Dec. 1	6.6	8.4	12.3	15.0	11.1	10.3
Dec. 29	49.7	2.4	14.3	13.1	10.8	10.4
1966						
Jan. 26	51.9	6.0	17.5	17.7	10.2	10.3
Feb. 23	25.6	7.5	15.9	13.7	10.4	10.3
March 23	22.8	9.6	25.9	27.4	9.8	9.8
April 20	51.3	14.7	17.3	11.6	10.6	10.9
May 18	5.6	26.3	12.9	12.5	10.4	10.6
June 22[a]	--	74.8	0.9	25.2	9.8	8.7
July 20	106.2	103.0	10.8	34.1	--	--
Aug. 17	91.7	--	9.7	8.8	--	--
Sept. 28	14.8	13.1	10.9	--	--	--

[a]Bay plankton consisted of a dense Aurelia aurita (Scyphozoan) population.

Figure 4. Representative chromatographic distributions of fatty acids extracted from the phytoplankton-microzooplankton (number 20 mesh net). Spatial and seasonal differences in composition of the plankton are seen by comparing the peak heights of corresponding fatty acids.

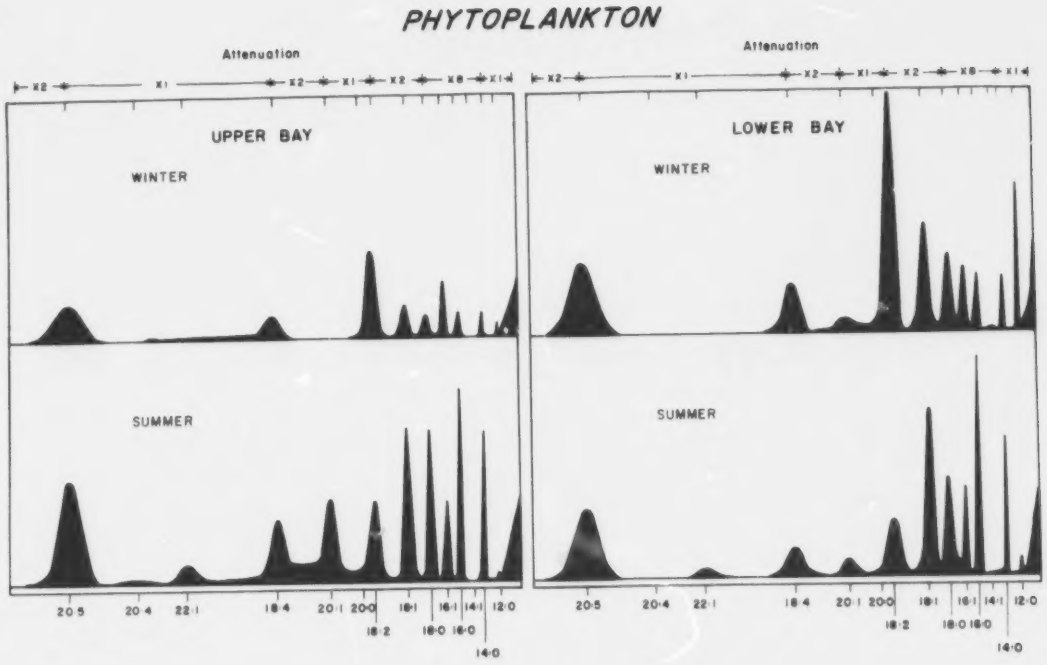
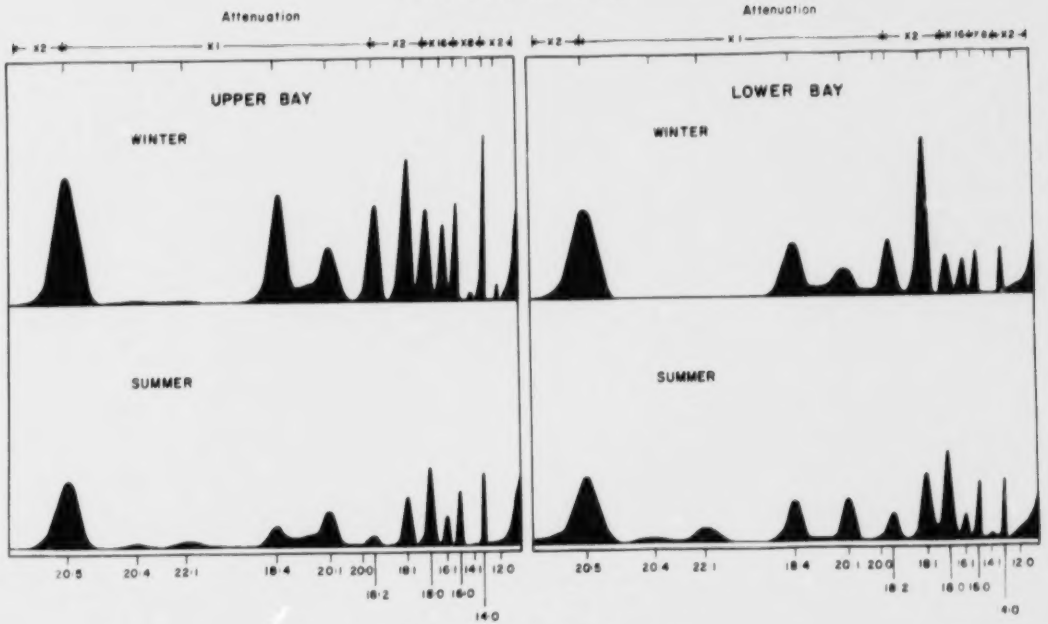

PHYTOPLANKTON
Attenuation
UPPER BAY
WINTER
SUMMER
Attenuation
LOWER BAY
WINTER
SUMMER
20:5
20:4
22:1
18:4
20:1
20:0
18:2
18:1
18:0
16:1
16:0
14:1
14:0
12:0
FATTY ACID METHYL ESTERS

Figure 5. Representative chromatographic distributions of fatty acids extracted from the zooplankton (number 2 mesh net) of Narragansett Bay.

ZOOPLANKTON



FATTY ACID METHYL ESTERS

third axis, extending backwards, through the plane of the paper. It helps the reader to imagine that he is looking into a box, the axes forming the edges, and in this box he sees a topographic surface. The ridges, valleys and hills are the levels and changes in fatty acid composition; they collectively portray a major biochemical sequence at the community level. Figure 6 shows the phytoplankton-zooplankton community at two stations, and Figure 7 is for the zooplankton.

The patterns in the Bay and Sound are similar. But the ocean community appears more stable in each case than the estuarine community. This agrees with what we know about the stability of plankton communities: ocean communities have greater order and structure than estuarine communities.

The phytoplankton-microzooplankton is considered first. During winter, the concentration of palmitoleic acid (16:1) increases from about 15% to 35% while palmitic acid (16:0) varies between 5 and 17%. Precipitous changes occur in May: palmitoleic acid drops from 35% to less than 10% of the total fatty acids and palmitic acid shoots upward, from 13% to 40%. Seasonal succession occurs in the phytoplankton. Dominance goes from a winter, diatom community to a summer, flagellate association (Figure 2). The two C_{16} acids then readjust from their extreme concentrations during succession. Palmitoleic acid levels off at approximately 15% when water temperatures reach the annual maximum in August. Palmitic acid decreases throughout the summer, falling below 20% in the fall. Eicosapentaenoic acid (20:5), a characteristic marine fatty acid (long-chain and highly unsaturated), diminishes during the spring

Figure 6. The fatty acid composition of the phytoplankton-microzooplankton at two stations: A, 12 miles within Narragansett Bay and B, Rhode Island Sound near the mouth of the Bay. Time and percent concentrations of the fatty acids are pictured as a three-dimensional surface. The fatty acids from 14:0 (myristic acid) to 20:5 (eicosapentaenoic acid) are arranged from front to rear according to increasing chromatographic retentions. November 1963 and January 1964 to January 1965.

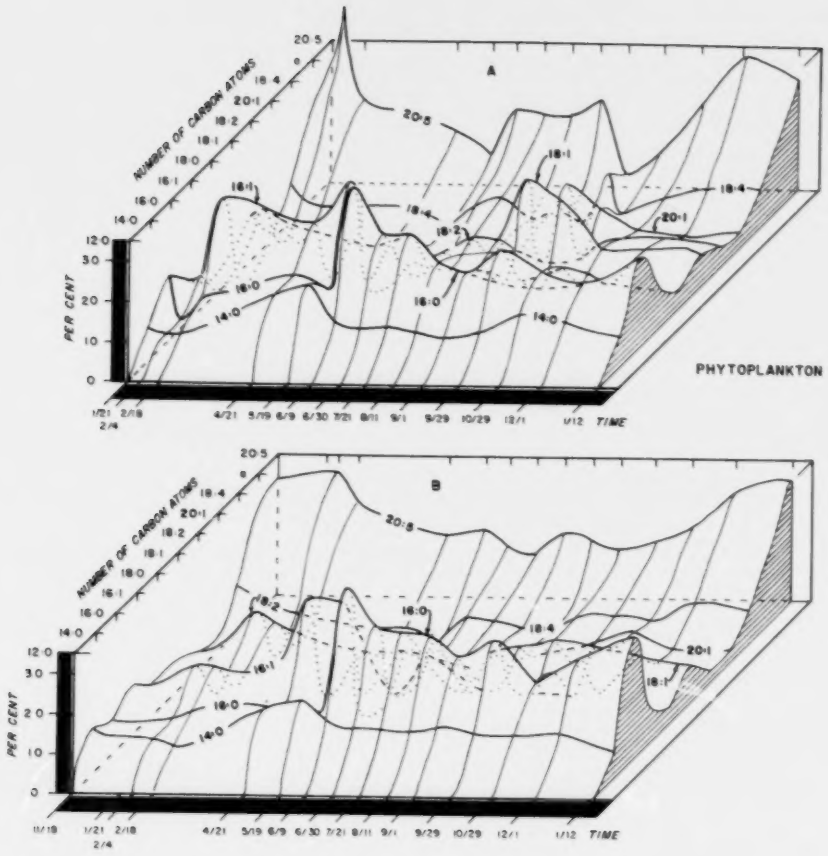
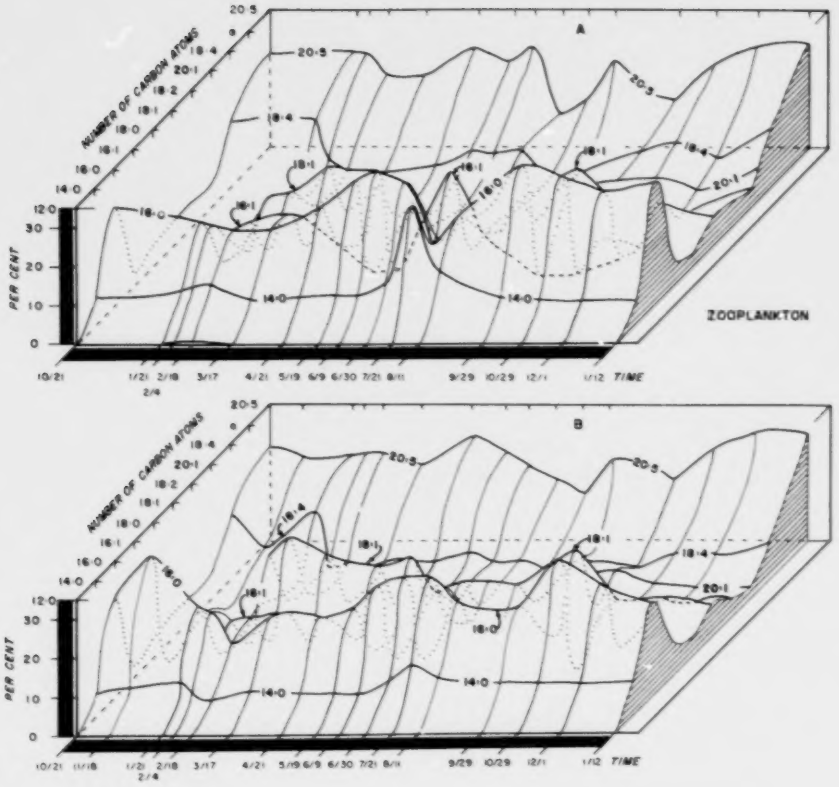

A
B
PHYTOPLANKTON
NUMBER OF CARBON ATOMS
PER CENT
TIME
20:5
18:4
20:1
18:2
18:1
18:0
16:1
16:0
14:0
12:0
3.0
2.0
1.0
0
11/19
1/21
2/4
2/18
4/21
5/19
6/9
6/30
7/21
8/11
9/1
9/29
10/29
12/1
1/12

Figure 7. The fatty acid composition of the zooplankton pictured as a three-dimensional surface.


A
B
NUMBER OF CARBON ATOMS
20:5
18:4
20:1
18:2
18:1
18:0
16:1
16:0
14:0
PER CENT
30
20
10
0
TIME
10/21
1/21
2/4
2/18
3/17
4/21
5/19
6/9
6/30
7/21
8/11
9/29
10/29
12/1
1/12
ZOOPLANKTON

and summer, reaching a low point in late August when the ctenephore *Mnemiopsis leidyi* is abundant. As temperatures cool in the fall, 20:5 increases to the winter level. The variations of this acid are, therefore, inversely related to water temperature. The other fatty acids have peak concentrations once or twice a year that are not related to progressive changes of the indigenous plankton.

The pattern of fatty acids in the zooplankton (Figure 7) also shows seasonal changes associated with vernal warming, autumnal cooling and the succession of species populations. During winter in the Bay and Sound, when the water is the coldest, palmitic acid (16:0) decreases while palmitoleic acid (16:1) rises quickly. A second pattern occurs in the C_{18} series: oleic acid (18:1) increases sharply while 18:4 decreases an equal amount. These variations may be associated with the arrival of the chaetognath *Sagitta elegans*, a migrant from coastal waters. As discussed later, the inverse relationships reflect the status of the indigenous community. The migratory chaetognath initiates major chemical changes in a zooplankton community.

Fatty acids also reflect annual variations in plankton production and the timing of cyclical events, as indicated in Table 2, where the mean seasonal concentrations are listed for two annual cycles. The analytical procedure was modified in the second sampling period, preserving the integrity of the long-chain acids from 24:0 to 26:1. Despite this artifact, real differences are apparent from season to season and from year to year.

Table 2. Fatty acid composition of the phytoplankton-microzooplankton community, weight percent of total methyl esters. Seasonal means for the winter-spring (W-S) and summer-fall (S-F) periods in Narragansett Bay and Rhode Island Sound.

Fatty acid	STATION AND YEAR							
	1963-64				1965-66			
	Bay		Sound		Bay		Sound	
	W-S	S-F	W-S	S-F	W-S	S-F	W-S	S-F
12:0	0.2	0.2	0.3	0.5	0.1	1.0	0.1	0.4
12:1					+	0.6	+	+
14:0	10.8	9.3	12.3	11.5	13.0	9.5	11.6	10.6
14:1	+	+	+	+	+	+	+	0.4
16:0	17.6	21.6	19.2	24.7	14.2	20.0	17.4	22.0
16:1[a]	18.6	13.4	19.3	14.0	15.8	8.6	11.3	10.0
16:3					3.1	1.2	2.3	0.9
18:0	5.1	6.7	5.1	6.9	3.2	4.9	5.3	3.1
18:1	8.1	14.0	8.4	10.5	10.0	6.0	17.3	11.7
18:2	8.0	3.8	8.7	4.3	7.0	3.9	3.2	4.1
18:4[c]	5.4	4.4	4.1	5.5	5.1	6.9	3.3	3.9
20:0[b]	+	+	+	+	0.2	0.2	0.3	+
20:1	1.3	6.6	1.5	3.9	3.8	4.6	5.8	4.1
22:0					0.3	0.4	+	+
22:1	0.7	2.9	0.4	1.0	2.9	1.5	2.5	1.9
20:4	+	+	+	+	+	+	0.2	0.3
20:5	23.1	15.3	20.9	18.1	12.1	14.2	10.8	12.1
24:0[d]					0	0	+	+
22:3					2.2	2.6	1.7	2.2
24:1					+	0.5	0.1	+
24:2					+	0.3	+	0.1
22:6					6.6	11.9	7.5	11.5
26:0					+	+	0	+
26:1					0.5	1.0	0.3	0.5

[a] includes 16:2

[b] includes 18:3

[c] includes 20:2

[d] identification tentative through 26:1. These acids were apparently oxidized in the laboratory during the 1963-64 periods.

Seasonally, the most noticeable change is an increase in the unsaturated fatty acids as temperature decreases. This is a well known phenomenon for single species (Lovern, 1964, Prosser, 1964, Allen, 1960), but all fatty acids in a community do not follow, as Tables 2 and 3 indicate. To be sure, the total percent concentration of all unsaturated acids through 20:5 is higher in the winter than in the summer. The differences between seasons range from 2.8% to 14.4% (phytoplankton-microzooplankton, Sound, 1963-64; zooplankton, Bay, 1965-66). But two acids -- 20:1 and 22:1 -- do not always behave in such a fashion, and the long-chain unsaturated acids from 22:3 to 26:1 are clearly more plentiful in summer than winter.

Annual differences cannot be explained at present. They are probably associated with the fact that plankton was generally more abundant and specific events in the annual cycle occurred earlier in 1964-65 than the following year. A rapid and sensitive means of determining annual trends is much in need: fatty acid variations may provide the key. This will be considered in phases 2 and 3 of the investigation.

Figures 8 and 9 emphasize the inverse relationships within the C_{16} and C_{18} series. In the Bay zooplankton, negative correlation coefficients are significant at the 0.5% level, and the significance level for the Sound zooplankton is 1.0% (1964-65).

As indicated previously, the reciprocal changes of 16:0 and 16:1 in the phytoplankton-microzooplankton are associated with seasonal succession of plant populations. An increase in one of these acids is usually matched by a decrease in the other. During

Table 3. Fatty acid composition of the zooplankton community, weight percent of total fatty acid methyl esters. Seasonal means and footnotes as in Table 2.

Fatty acid	YEAR, STATION AND PERIOD							
	1963-64				1965-66			
	Bay		Sound		Bay		Sound	
	W-S	S-F	W-S	S-F	W-S	S-F	W-S	S-F
12:0	+	+	+	+	0.2	0.4	0.2	0.2
12:1					+	+	+	+
14:0	8.0	10.6	6.5	8.4	7.5	5.6	7.0	12.2
14:1	+	+	+	+	1.0	0.4	0.1	0.6
16:0	25.1	27.5	23.1	30.3	15.4	25.6	18.9	18.7
16:1[a]	10.0	10.3	10.9	8.1	12.0	6.0	8.8	8.5
16:3					2.3	0.2	3.0	1.5
18:0	5.1	7.6	4.5	7.3	4.7	6.1	3.2	3.3
18:1	11.6	10.4	17.0	14.8	12.3	8.8	16.3	10.1
18:2	4.6	4.1	4.7	3.1	4.1	2.5	3.9	2.8
20:0[b]	+	+	+	+	+	+	+	+
20:1	3.7	5.0	3.2	5.5	3.8	4.8	3.2	3.8
18:4[c]	8.8	8.8	7.0	4.5	5.6	5.5	4.5	5.6
22:0					0.3	0.6	0.2	0.5
22:1	0.3	0.6	0.4	0.8	1.6	1.2	0.8	3.3
20:4	+	+	+	+	+	0.6	0.3	0.4
20:5	23.1	15.4	22.9	17.2	14.5	12.8	16.6	13.9
24:0[d]					+	+	+	+
22:3					2.6	2.8	2.0	2.7
24:1					+	0.5	0.3	0.7
24:2					+	0.4	+	+
22:6					11.7	14.2	8.1	9.5
26:0					+	+	+	+
26:1					0.2	1.0	0.3	0.8

Figure 8. The inverse relationships of palmitic acid (16:0) and palmitoleic acid (16:1) concentrations in the phytoplankton-microzooplankton and zooplankton of Narragansett Bay and Rhode Island Sound.

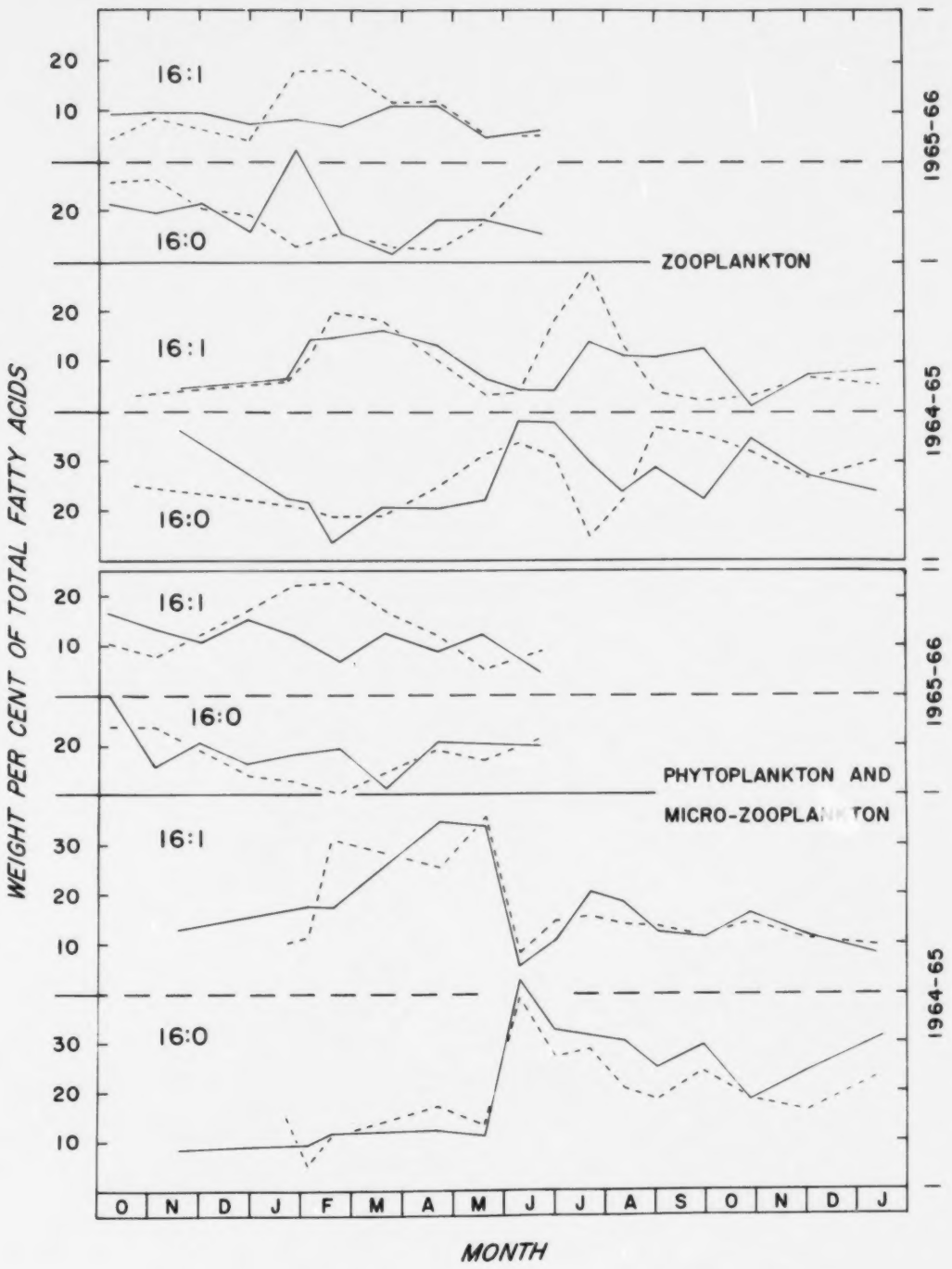
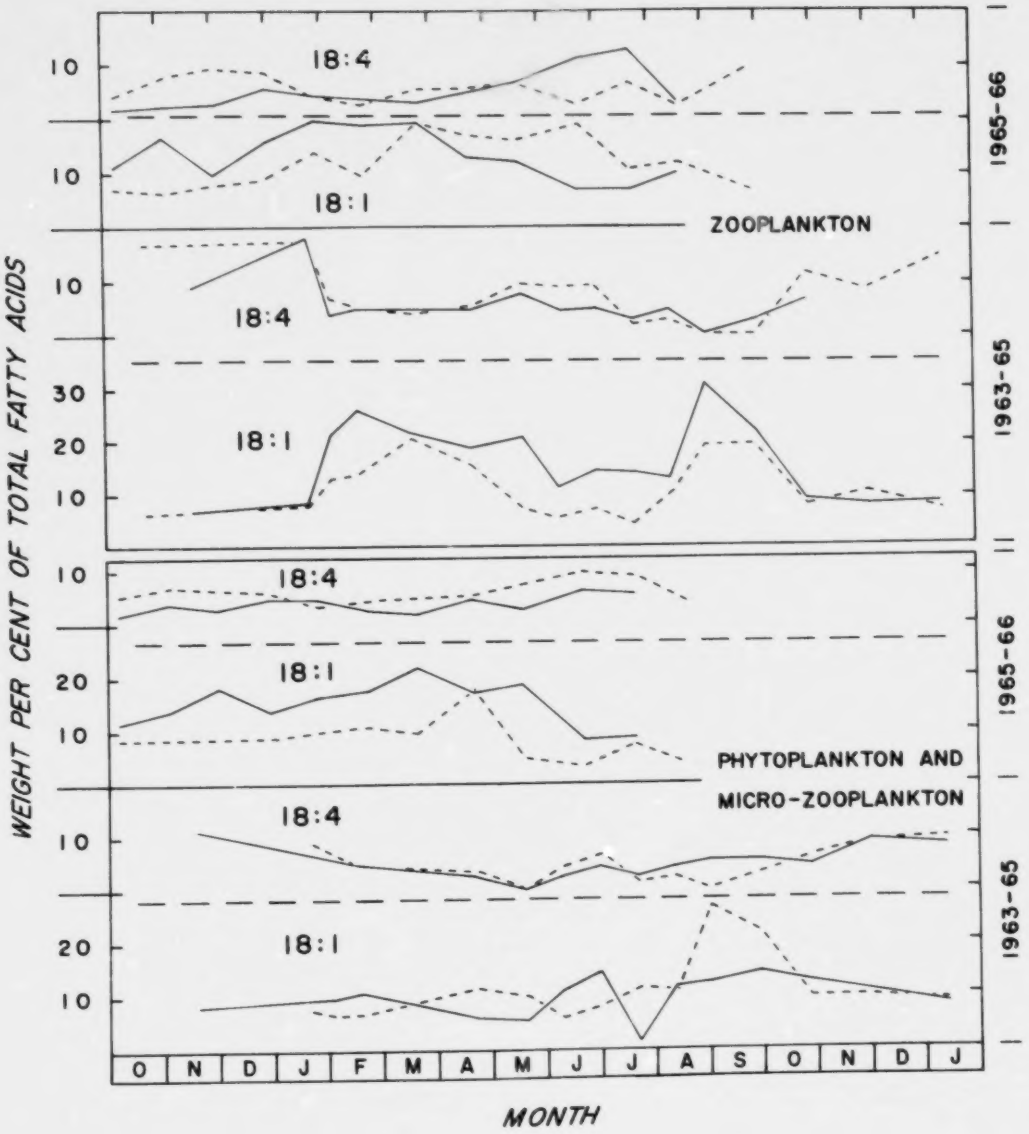

16:1
16:0
ZOOPLANKTON
1965-66
1964-65
PHYTOPLANKTON AND
MICRO-ZOOPLANKTON
WEIGHT PER CENT OF TOTAL FATTY ACIDS
O N D J F M A M J J A S O N D J
MONTH

Figure 9. Relationships between oleic acid (18:1) and stearidonic acid (18:4, including 20:2) in the plankton of Narragansett Bay and Rhode Island Sound.


WEIGHT PER CENT OF TOTAL FATTY ACIDS
18:4
18:1
ZOOPLANKTON
PHYTOPLANKTON AND
MICRO-ZOOPLANKTON
1965-66
1963-65
1965-66
1963-65
10
20
30
O N D J F M A M J J A S O N D J
MONTH

the second year of this study, the pattern occurred in the Bay, but in Rhode Island Sound, at a station well away from the immediate area of Bay influence, inverse relationship was not obvious. The pattern of reciprocal changes occured at both stations the previous year, but at this time the Sound station was located near the mouth of Narragansett Bay.

The annual cycle of zooplankton production is also associated with the 16:0-16:1 relationship. Trends of change in the weight ratio of these acids reflect the condition of the zooplankton. The 16:0/16:1 ratio is 3.5 in January, 1965, and it decreases through February. The zooplankton is now a boreal community, living under ideal temperature conditions. During vernal warming (March-June) the boreal community is subjected to environmental stress, and the ratio increases from 1.03 to 8.5. When summer begins, the ratio drops sharply to 0.52 (July). By this time *Acartia tonsa,* the dominant summer copepod in the Bay, has produced a young and healthy population. But the ratio soon increases again, signifying adverse conditions resulting from depleted food supplies and August temperatures. The ratio continues to increase despite the appearance of *Mnemiopsis leidyi,* a ctenephore predator which crops the copepods drastically. In late September the ratio reaches 13.3, the high point for the year. The ensuing decreases are variable, but by the time the annual cycle begins in winter, intermediate values have been re-established, and the boreal community augments again.

The behavior of the C_{16} and C_{18} acids indicates cyclical stresses which are compensated first by internal responses and then

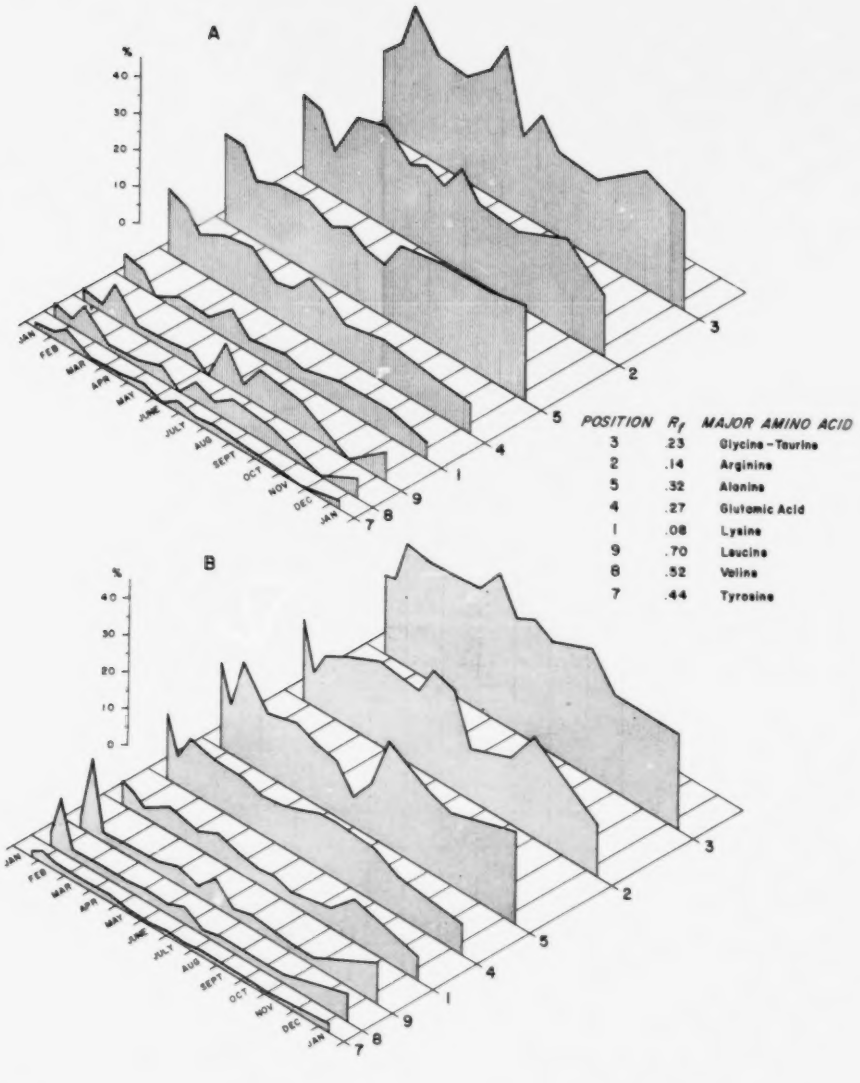
by the species structure of the community. When the 16:0/16:1 ratio is decreasing, the zooplankton community is young and robust; an increasing ratio signifies death and seasonal succession. These patterns confer a sense of chemical realism on the concept of organization at the community level. The biochemical integrity of a plankton community is real despite the ever changing nature of the associations. Chemical composition varies in time and space but not in a random manner. The composition follows patterns that are interrelated with each other, with seasonal succession and with the controlling factors of the environment.

C. Internal Responses: distributions of the free amino acids.

The seasonal distribution of free amino acids in the zooplankton is shown in Figure 10. The analyses were one-dimensional paper chromatograms developed with butanol:acetic acid:water (60:15:25) and scanned with a recording densitometer. The most marked changes take place when the summer species replace the winter species -- the May, June and July period. In this respect the free amino acids resemble the fatty acids. Another period of change is in February, when *Sagitta elegans* enters the area. As is the case for the fatty acids, the amino acid spectrum appears less variable in the Sound (B) than in the Bay (A).

Seasonal data are given in Table 4. These analyses were made by liquid column chromatography. In the phytoplankton-microzooplankton community, the mean total concentration of all amino acids is obviously higher in summer than winter by at least 200 μM/g dry weight. With the exception of only two amino acids occurring in

Figure 10. Free amino acid patterns in the zooplankton at two stations: A, 12 miles within Narragansett Bay and B, near the mouth of the Bay in Rhode Island Sound. January 1964 to January 1965.



POSITION	R_f	MAJOR AMINO ACID
3	.23	Glycine – Taurine
2	.14	Arginine
5	.32	Alanine
4	.27	Glutamic Acid
1	.08	Lysine
9	.70	Leucine
8	.52	Valine
7	.44	Tyrosine

Table 4. Mean seasonal concentrations (µM/g dry weight) of free amino acids in the plankton communities of Narragansett Bay and Rhode Island Sound. 1965-66.

Amino acid	COMMUNITY, STATION AND PERIOD							
	Phytoplankton				Zooplankton			
	Bay		Sound		Bay		Sound	
	W-S	S-F	W-S	S-F	W-S	S-F	W-S	S-F
CYST	1.5	0.8	2.6	2.7	3.6	2.3	2.4	3.4
TAU	18.2	34.3	31.9	33.4	60.7	87.0	66.4	66.1
ASP	2.5	3.0	3.9	8.4	5.9	3.8	4.2	6.1
THR	4.8	8.3	8.0	15.7	16.2	11.4	11.9	17.6
SER	4.3	6.0	5.8	13.5	10.5	10.9	9.6	11.9
HSER	2.1	4.5	5.2	5.3	7.4	9.8	8.8	5.8
GLU	8.0	11.9	8.1	6.8	18.0	14.2	10.9	12.9
PRO	28.8	40.1	32.2	21.4	81.0	87.1	78.1	34.0
GLY	40.4	76.0	69.7	161.8	124.8	179.3	153.7	128.9
ALA	18.8	33.6	27.3	47.5	59.4	59.7	39.0	45.2
CYS	-	0.3	-	-	-	0.2	-	-
VAL	4.0	6.2	6.4	15.3	15.8	12.1	13.3	16.7
MET	1.9	2.5	2.5	6.3	6.6	5.5	5.1	6.0
ILEU	2.5	3.6	4.1	10.7	10.8	7.6	8.6	10.4
LEU	4.6	7.1	7.1	19.8	18.8	13.8	15.1	18.3
TYR	2.4	4.3	3.6	9.6	10.2	8.2	6.8	8.8
PHE	3.4	3.7	4.0	7.4	11.0	6.9	7.1	6.7
OLYS	0.1	0.1	0.4	0.7	0.6	0.1	0.1	-
ORN	0.2	1.6	0.5	1.7	1.0	1.6	0.4	1.1
LYS	6.0	8.8	8.6	19.7	18.8	17.7	15.9	16.6
HIST	0.3	2.3	0.9	3.7	4.1	4.5	3.1	5.0
TRYP	0.2	0.5	0.3	+	1.7	0.9	0.9	+
ARG	13.3	24.9	16.6	45.4	42.8	52.5	37.2	54.8
TOTAL $\bar{X}$	136.8	284.3	249.4	456.5	529.8	597.1	498.7	352.5

low concentrations, all amino acids reflect this seasonal increase in the Bay. During the winter-spring period, diatoms low in free amino acids predominate, at least in the Bay community, but in the summer-fall period, the developmental stages of copepods and other animals contribute heavily to the concentrations of the free amino acids. Two important amino acids in the Sound -- glutamic acid and proline -- decrease during the summer-fall period. Since the size fraction represented here is an important food source for many animals, it is important to note the marked differences in average composition.

In the zooplankton, total concentrations in the Bay are similar summer and winter, but in the Sound the summer mean is low, due to proline and glycine. The other amino acids generally increase in summer.

Responses of the free amino acids to environmental change can be considered from the standpoint of correlated changes within the pool. Concentrations of each amino acid during the observation period are compared with each of the other amino acids by means of correlation coefficients. The coefficients are arranged in matrix, one matrix for each station and community. In Tables 5-8, the amino acids are arbitrarily arranged in the matrices according to their order of elution from the chromatographic column. The correlation coefficients indicate varying degrees of correspondence between all possible pairs as they vary in concentration during the observation period. Coefficients significant at the 5% level are underlined, and groups of 4 or more significant coefficients are blocked out.

TABLE 5.

Matrix of correlation coefficients for free amino acid concentrations (μM/g dry tissue) in zooplankton from Narragansett Bay, Rhode Island. October, 1965 to May, 1966.

		1 CYST	2 TAU	3 ASP	4 THR	5 SER	6 GLU	7 PRO	8 GLY	9 ALA	10 VAL	11 MET	12 ILEU	13 LEU	14 TYR	15 PHE	16 LYS	17 HIS	18 ARG
1	CYST	1.00																	
2	TAU	-0.17	1.00																
3	ASP	0.76	-0.16	1.00															
4	THR	0.40	-0.30	0.42	1.00														
5	SER	0.55	0.02	0.36	0.80	1.00													
6	GLU	-0.23	0.35	-0.05	0.11	-0.04	1.00												
7	PRO	-0.18	0.74	-0.26	-0.32	0.01	0.64	1.00											
8	GLY	-0.09	0.97	-0.21	-0.37	-0.00	0.28	0.77	1.00										
9	ALA	0.38	0.59	0.16	0.23	0.51	0.59	0.75	0.61	1.00									
10	VAL	0.49	-0.07	0.57	0.83	0.65	0.45	0.05	-0.10	0.52	1.00								
11	MET	0.67	-0.25	0.61	0.89	0.79	0.23	-0.09	-0.26	0.48	0.93	1.00							
12	ILEU	0.41	-0.09	0.53	0.74	0.55	0.57	0.16	-0.12	0.54	0.97	0.89	1.00						
13	LEU	0.55	-0.21	0.64	0.79	0.60	0.43	0.01	-0.24	0.47	0.97	0.95	0.98	1.00					
14	TYR	0.42	-0.24	0.49	0.54	0.39	0.57	0.22	-0.24	0.49	0.83	0.79	0.93	0.92	1.00				
15	PHE	0.28	-0.29	0.50	0.42	0.11	0.46	0.04	-0.32	0.25	0.66	0.61	0.77	0.76	0.82	1.00			
16	LYS	0.60	-0.03	0.58	0.90	0.91	0.17	-0.02	-0.08	0.53	0.89	0.94	0.81	0.85	0.64	0.45	1.00		
17	HIS	0.20	0.38	0.31	-0.05	0.00	0.69	0.71	0.42	0.66	0.49	0.30	0.61	0.52	0.70	0.58	0.23	1.00	
18	ARG	0.15	0.88	-0.12	-0.08	0.36	0.24	0.72	0.91	0.80	0.08	0.03	0.03	-0.05	-0.11	-0.28	0.22	0.33	1.00

TABLE 6.

Matrix of correlation coefficients for free amino acid concentrations (μM/g dry tissue) in zooplankton from Rhode Island Sound. October, 1965 to June, 1966.

		1 CYST	2 TAU	3 ASP	4 THR	5 SER	6 GLU	7 PRO	8 GLY	9 ALA	10 VAL	11 MET	12 ILEU	13 LEU	14 TYR	15 PHE	16 LYS	17 HIS	18 ARG
1	CYST	1.00																	
2	TAU	0.52	1.00																
3	ASP	0.91	0.38	1.00															
4	THR	0.85	0.35	0.88	1.00														
5	SER	0.81	0.31	0.77	0.85	1.00													
6	GLU	0.76	0.43	0.75	0.78	0.51	1.00												
7	PRO	0.08	0.59	-0.08	0.10	-0.02	0.04	1.00											
8	GLY	0.25	0.62	0.16	0.22	0.21	0.08	0.82	1.00										
9	ALA	0.83	0.69	0.77	0.73	0.79	0.54	0.23	0.57	1.00									
10	VAL	0.76	0.25	0.85	0.84	0.87	0.48	-0.09	0.09	0.71	1.00								
11	MET	0.74	0.21	0.83	0.82	0.89	0.44	-0.07	0.17	0.73	0.98	1.00							
12	ILEU	0.78	0.27	0.89	0.87	0.87	0.55	-0.05	0.16	0.73	0.99	0.98	1.00						
13	LEU	0.74	0.25	0.85	0.81	0.86	0.45	-0.10	0.13	0.71	0.99	0.99	0.99	1.00					
14	TYR	0.86	0.41	0.87	0.80	0.92	0.48	-0.07	0.19	0.81	0.92	0.90	0.91	0.92	1.00				
15	PHE	0.48	0.11	0.60	0.52	0.64	0.19	-0.05	0.13	0.52	0.86	0.89	0.86	0.89	0.70	1.00			
16	LYS	0.72	0.32	0.82	0.85	0.86	0.50	0.05	0.30	0.79	0.96	0.97	0.97	0.96	0.85	0.86	1.00		
17	HIS	0.81	0.57	0.88	0.87	0.67	0.73	0.13	0.30	0.83	0.78	0.74	0.80	0.75	0.74	0.51	0.81	1.00	
18	ARG	0.27	-0.10	0.23	-0.02	0.25	-0.20	-0.12	0.30	0.33	0.12	0.24	0.15	0.18	0.32	0.21	0.14	-0.06	1.00

TABLE 7.

Matrix of correlation coefficients for free amino acid concentrations (μM/g dry tissue) in phytoplankton-microzooplankton from Narragansett Bay, Rhode Island. October, 1965 to June, 1966.

		1 CYST	2 TAU	3 ASP	4 THR	5 SER	6 GLU	7 PRO	8 GLY	9 ALA	10 VAL	11 MET	12 ILEU	13 LEU	14 TYR	15 PHE	16 LYS	17 HIS	18 ARG
1	CYST	1.00																	
2	TAU	0.47	1.00																
3	ASP	0.32	0.73	1.00															
4	THR	0.19	0.92	0.83	1.00														
5	SER	0.18	0.91	0.77	0.98	1.00													
6	GLU	0.31	0.80	0.58	0.77	0.70	1.00												
7	PRO	0.33	0.97	0.77	0.97	0.96	0.86	1.00											
8	GLY	0.32	0.93	0.74	0.91	0.92	0.87	0.98	1.00										
9	ALA	0.29	0.93	0.77	0.95	0.96	0.83	0.99	0.99	1.00									
10	VAL	0.20	0.92	0.81	0.98	0.99	0.69	0.96	0.92	0.96	1.00								
11	MET	0.22	0.92	0.77	0.97	0.99	0.63	0.93	0.88	0.92	0.99	1.00							
12	ILEU	0.21	0.93	0.81	0.98	0.99	0.70	0.96	0.92	0.96	0.99	0.99	1.00						
13	LEU	0.15	0.91	0.76	0.97	0.99	0.72	0.96	0.94	0.97	0.99	0.98	0.99	1.00					
14	TYR	0.30	0.95	0.83	0.98	0.98	0.73	0.97	0.93	0.96	0.99	0.99	0.99	0.98	1.00				
15	PHE	0.15	0.83	0.75	0.92	0.93	0.48	0.83	0.74	0.81	0.94	0.97	0.94	0.91	0.93	1.00			
16	LYS	0.17	0.90	0.79	0.98	0.99	0.64	0.93	0.88	0.93	0.99	0.99	0.99	0.98	0.98	0.97	1.00		
17	HIS	0.61	0.78	0.46	0.60	0.56	0.92	0.76	0.79	0.73	0.55	0.50	0.57	0.57	0.62	0.33	0.49	1.00	
18	ARG	0.26	0.94	0.76	0.95	0.97	0.81	0.99	0.99	0.99	0.97	0.94	0.97	0.98	0.97	0.83	0.94	0.71	1.00

TABLE 8.

Matrix of correlation coefficients for free amino acid concentrations (μM/g dry tissue) in phytoplankton-microzooplankton from Rhode Island Sound. October, 1965 to June, 1966.

		1 CYST	2 TAU	3 ASP	4 THR	5 SER	6 GLU	7 PRO	8 GLY	9 ALA	10 VAL	11 MET	12 ILEU	13 LEU	14 TYR	15 PHE	16 LYS	17 HIS	18 ARG
1	CYST	1.00																	
2	TAU	0.89	1.00																
3	ASP	0.78	0.71	1.00															
4	THR	0.86	0.78	0.93	1.00														
5	SER	0.86	0.82	0.79	0.93	1.00													
6	GLU	0.82	0.59	0.68	0.69	0.53	1.00												
7	PRO	0.92	0.83	0.70	0.71	0.66	0.90	1.00											
8	GLY	0.63	0.75	0.79	0.81	0.85	0.22	0.42	1.00										
9	ALA	0.90	0.86	0.94	0.96	0.84	0.80	0.84	0.74	1.00									
10	VAL	0.90	0.79	0.85	0.94	0.96	0.67	0.77	0.79	0.88	1.00								
11	MET	0.29	0.34	0.46	0.61	0.72	-0.10	-0.04	0.76	0.39	0.56	1.00							
12	ILEU	0.87	0.80	0.87	0.94	0.96	0.65	0.75	0.81	0.88	0.99	0.59	1.00						
13	LEU	0.87	0.80	0.86	0.92	0.95	0.64	0.80	0.82	0.87	0.99	0.57	0.99	1.00					
14	TYR	0.67	0.68	0.72	0.87	0.95	0.30	0.41	0.89	0.72	0.86	0.89	0.87	0.86	1.00				
15	PHE	0.71	0.55	0.69	0.88	0.92	0.51	0.49	0.67	0.72	0.88	0.76	0.88	0.86	0.92	1.00			
16	LYS	0.90	0.73	0.73	0.73	0.68	0.90	0.97	0.43	0.81	0.83	0.01	0.81	0.82	0.45	0.57	1.00		
17	HIS	0.87	0.96	0.66	0.71	0.73	0.69	0.91	0.61	0.83	0.76	0.14	0.74	0.75	0.54	0.48	0.83	1.00	
18	ARG	0.75	0.70	0.86	0.88	0.89	0.47	0.60	0.90	0.80	0.93	0.63	0.95	0.96	0.85	0.78	0.69	0.63	1.00

A matrix can be rotated by factor analysis so that blocks of communality are formed along the diagonal, but for present purposes it is sufficient to compare the relative sizes of corresponding blocks in each of the four matrices. Compare the blocks in Tables 5 and 6, for example. Table 5 shows three small blocks, whereas the corresponding blocks in Table 6 are considerably larger. This means that the free amino acid pool in the Bay zooplankton community lacks coordination: the components vary randomly with respect to one another; a change in the concentration of one amino acid does not usually correspond to changes of the other amino acids. But in the Sound, the large blocks of interrelated change signify, once again, that the community has achieved biochemical integrity.

One large block with several small "windows" of nonsignificant coefficients is shown for the phytoplankton-microzooplankton of the Bay (Table 7). The same general pattern is indicated for the Sound, but the windows are larger and cysteic acid is included in the block. We are not able to explain the high degree of chemical correspondence in the Bay community. This size group consists of varying proportions of plant and animal plankton.

These matrices are pictures of the metabolic balance struck within a community -- formation and accumulation balanced against utilization and excretion. As such, the blocks are characteristic finger prints of the environment's ability to produce under varying situations. It follows that a change in the quality of the environment should be apparent from a change in the fingerprint. The fingerprints should reveal the way the environment changes or is altered by various factors including pollutants. When more is

known about these pictures, and they are reduced by canonical analysis and factor analysis to simpler terms, we should be able to pinpoint the origins of environmental change and predict the long-term effects on the biota.

IV. The Effects of Incinerator Residues on the Fatty Acid Composition of the Winter Flounder (*Pseudopleuronectes americanus*).

A. Relevance.

The city of Boston is considering a plan to dispose of municipal incinerator waste in the sea. Silverman (1964) and First (1966) described the program. At present, the incinerator wastes are used for land fill, but the refuse could be incinerated aboard ships and then released in approved offshore areas. With support from the Public Health Service, the Harvard School of Public Health is investigating the effects of incinerator residue on marine organisms. One of these studies conducted by Dr. Candace Oviatt in Narragansett Bay deals with mortality of the winter flounder.

B. Mortality experiments.

Two pens enclosing 100 square feet of bottom were placed in shallow water. One pen was kept as a control and a slurry of sieved incinerator waste was added to the other. The sieved residue comprised 35-50% of the total combusted residue and had a mean diameter of 3.5 mm. An atomic absorption analysis of the acid soluble fraction made at the National Marine Water Quality Laboratory (Kingston, R.I.) showed that the most important elements were Al, Fe, Ca, Na and Zn. The slurry consisted of four pounds of ash mixed with water, and it was placed in the experimental pen

on Tuesday and Friday of every week. This quantity of ash was twice the amount that would be dumped offshore in the approved areas (500 tons over one square mile).

Seventy-five flounder were added to each pen in March 1966 and removed to the laboratory nine months later to avoid the freeze. The flounders were then held in running sea water until January, 1967.

Throughout the experiment the mortality rates in the control and treatment pens were the same. No toxic effect could be ascribed to the ash treatment under the conditions of this experiment.

C. Response hypothesis and preliminary results.

It was hypothesized that the flounders may have responded to the ash even though there was no indication from mortality rates. Secondly, an important response would be revealed by the fatty acid spectra, and finally, a major internal change would eventually lead to exhaustion and death.

The results of fatty acid analysis on the liver and testes shown in Tables 9 and 10 do not support the hypothesis. The mean concentrations of all fatty acids in the control and ash treatment groups are similar.

This is a preliminary experiment, and the results are not conclusive. High mortality in both the control and treatment groups apparently resulted from a fin rot infection associated with confinement of the fish in pens. The stress of this infection could override metabolic responses to the ash treatment.

Table 9. Fatty acid composition of flounder (Pseudopleuronectes americanus) liver in weight percent of total methyl esters. The treatment group was exposed to sieved incinerator residue for nine months.

Fatty Acid	Individuals and means									
	Control						Treatment			
	1	2	5	9	10	$\bar{X}$	6	7	8	$\bar{X}$
12:0	+	+	+	+	+	+	+	0.4	+	0.1
12:1	+	+	+	+	+	+	+	+	+	+
14:0	1.5	1.9	2.0	4.1	2.2	2.3	4.0	2.9	2.8	3.2
14:1	0.9	2.0	0.4	0.4	0.3	0.8	0.7	0.4	0.6	0.6
16:0	26.3	15.4	19.2	30.3	20.6	22.4	24.7	20.0	25.2	23.3
16:1	8.7	15.8	8.1	7.4	15.4	11.1	7.7	12.2	10.6	10.2
16:2	+	+	3.0	+	2.6	1.1	+	+	2.1	0.7
16:3	1.3	0.8	1.5	+	1.3	1.0	0	+	1.0	0.3
18:0	3.6	0	3.6	4.8	2.4	2.9	4.6	3.4	4.4	4.1
18:1	16.4	23.2	14.6	11.8	21.0	17.4	18.5	12.5	11.6	14.2
18:2	1.5	1.2	1.8	1.5	0.5	1.3	1.4	1.4	2.3	1.7
20:0	+	0	+	+	+	+	+	0.8	+	0.3
20:1	1.9	5.7	3.7	2.0	3.9	3.4	3.8	3.1	3.3	3.4
18:4	+	+	+	+	+	+	+	+	+	+
20:2	+	+	+	+	+	+	+	+	+	+
22:0	+	+	+	+	+	+	+	+	+	+
22:1	5.5	1.5	5.7	6.0	5.0	4.7	3.7	5.8	7.3	5.6
20:4	+	+	1.6	+	1.8	0.7	1.2	+	+	0.4
20:5	14.6	11.9	10.4	10.2	7.6	10.9	12.0	10.2	10.4	10.9
24:0	+	+	+	+	+	+	+	+	+	+
22:3	1.8	3.0	2.7	3.3	2.1	2.6	1.8	1.9	2.2	2.0
24:1	1.3	0.5	1.9	2.1	1.2	1.4	1.5	1.5	1.1	1.4
24:2	2.7	4.0	1.9	1.9	1.2	2.3	2.8	1.9	2.6	2.4
22:6	10.3	10.9	14.7	10.5	8.5	11.0	9.6	19.1	10.4	13.0
26:0	+	+	+	+	+	+	+	+	+	+
26:1	1.8	2.3	3.5	3.3	2.4	2.7	2.1	2.1	2.2	2.1

Date	1/17/67	1/17/67	1/19/67	1/20/67	1/20/67	1/19/67	1/20/67	1/20/67
Sex	M	F	F	M	M	M	F	M
Stage	mat.	mat.	immat.	mat.	immat.	mat.	immat.	mat.
Wt.	184g	210g	172g	168g	115g	130g	124g	102g
Lgth	24cm	22cm	22.5cm	22.8cm	19cm	20.3cm	20.3cm	17.5cm
Para-sites	no	no	no	no	+	+	+++	no
Liver wt.	3.94g	4.66g	3.18g	2.25	2.33	2.07g	1.67g	1.18
$\%H_2O$ in Liver	77.92	74.03	74.49	76.91	73.84	79.71	74.96	76.71

Table 10. Fatty acid composition of pooled testes samples from the winter flounder (Pseudopleuronectes americanus). Weight percent of total fatty acid methyl esters.

	Testes	
	Control Flounder I & IX	Treatment Flounder VI & VIII
12:0	+	+
12:1	0.1	0.2
14:0	0.8	0.9
14:1	0.8	0.4
16:0	17.4	22.3
16:1	4.8	5.9
16:2	+	+
16:3	+	+
18:0	3.5	2.4
18:1	27.9	26.1
18:2	2.3	1.7
20:0	+	+
20:1	3.7	2.8
18:4	+	+
20:2	+	+
22:0	+	+
22:1	4.9	3.6
20:5	8.4	8.9
24:0	+	+
22:3	2.5	2.3
24:1	1.0	1.0
24:2	4.3	4.9
22:6	12.9	12.6
26:0	+	+
26:1	4.5	3.8

	I	IX	VI	VIII
Date	1/17/67	1/20/67	1/19/67	1/20/67
Stage	mature	mature	mature	mature
Weight	184g	168g	130g	102g
Length	24cm	22.8cm	20.3cm	17.5cm
Parasites	no	no	+	no
Testes wt.	30.46g	16.62g	9.15g	9.81g
% H_2O in Testes	81.5%	86.07%	81.7%	85.6%

Attempts are being made to control the infection. We plan to duplicate the experiment under better conditions and to measure the responses of serum proteins and amino acids along with the fatty acids. It should then be possible to state the validity of this hypothesis: high treatment levels cause an internal response that is not apparent from mortality estimates but, if allowed to continue, may be harmful. The internal response could then be used to monitor the field trials of a disposal program. If the load became dangerous, the population's internal responses would reveal the condition far enough in advance to avert mortality. These responses are symptoms, a presage or warning of foreboding conditions that can be alleviated if action is taken soon enough.

V. Chemical Specificity of Mollusks in Terms of Environmental History and Genetic Influences.

Up to this point the chemical responses of marine organisms have been considered in terms of succession and environmental control. The primary mechanism of chemical specificity is, of course, genetic. It is appropriate to consider the interaction of genetic influences and environmental history on chemical composition and response.

Physiological species occur in mollusks. Spawning temperatures, for example, differ in northern and southern populations, and metabolic rates are adapted to local conditions. However, neither spawning temperatures nor rate adjusted metabolism curves are sufficiently sensitive to reveal genetic differences between populations separated by short distances. Fatty acid distributions can be applied to this problem as a test of the sensitivity of the approach.

Two preliminary sets of experiments have been conducted, one in 1964 and the other in 1965. Now that adequate facilities are available to hold marine organisms under natural conditions for long periods, the experiments will be duplicated during summer, 1967.

The mollusks were collected in Narragansett Bay and in Pt. Judith Pond, a small embayment with a narrow opening to the sea, 9 miles west of the Bay. The mollusks were placed in boxes which were set on the bottom in the West Passage of Narragansett Bay (salinity 30-32 °/oo). In this manner the environmental influences were kept the same on stocks from two contrasting habitats.

Results of the first series of observations (1964) are shown in Table 11. After 3-1/2 months in Narragansett Bay, the hard clam *(Mercenaria mercenaria)* from Pt. Judith Pond differred in fatty acid composition from clams native to Narragansett Bay. The Pt. Judith specimens had higher relative concentrations of 14:0 and 16:1, but less 18:0 than those from the Bay. In addition, a small but distinctive qualitative difference occurred on the chromatograms between the 20:1 and 18:4 peaks. The Bay specimens had a shoulder on the 18:4 peak, an unresolved component which was absent in the Pond clams. The shoulder is indicated in Figure 11, chromatograms A and B, but it is absent in the Pond clams, chromatograms C and D.

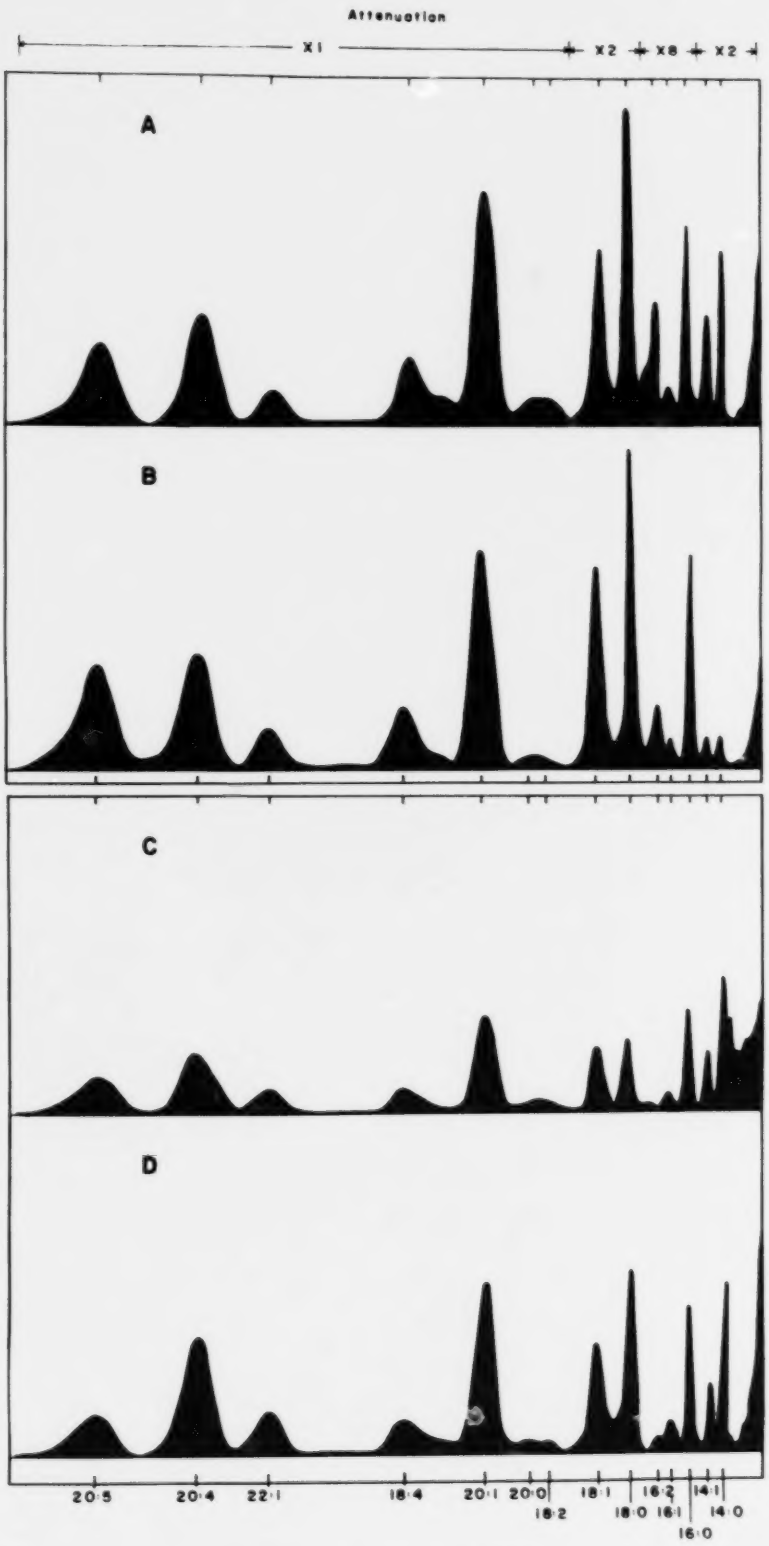
Another difference is the prevalence of short-chain acids (less than C_{12}) in the Pt. Judith Pond specimens. Note in Figure 11, C and D, that the solvent front at the right hand margin is high and irregular. This results from low molecular weight compounds partitioned with the solvent. These components can be separated by temperature programming during the chromatographic analysis.

Table II. Fatty acid composition of mollusks collected in Pt. Judith Pond and Narragansett Bay in May, 1964. The specimens were held for 3-1/2 months in Narragansett Bay before analysis, except for numbers 1-4, which were held in the Bay for 1 month.

Species	Location	No.	FATTY ACIDS -- WEIGHT PER CENT													
			14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	20:0	20:1	18:4	22:1	20:4	20:5
Mercenaria mercenaria	Lower Pond	2	2.8	1.9	19.6	8.9[a]	nr	9.4	11.1	--	2.2	--	13.8	4.6	4.1	7.8	13.7
		6	2.6	1.3	26.3	9.1[a]	+	10.8	9.7	+	+		13.1	3.1	4.5	13.6	6.0
		7	3.2	1.8	30.5	8.1[a]	+	7.5	9.8	+	+		12.5	4.2	3.0	11.6	8.0
	Upper Pond (polluted)	3	4.2	1.1	25.4	8.6	6.3	6.8	8.6	+	+		8.8	4.2	2.7	9.2	14.0
	Bay	8	1.4	2.4	27.9	3.8[a]	2.5	11.3	11.8	+	+		10.3	4.1	2.7	9.2	9.3
		9	1.9	1.5	24.7	6.5[a]	5.6	13.4	10.9	+	+		12.8	4.4	2.5	8.6	7.1
Mya arenaria	Pond	4	4.6	1.1	27.5	13.2	1.2	4.1	10.8	nr	2.6		15.0	5.2	1.8	+	12.9
		10	4.7	1.0	28.7	10.9	1.3	5.0	10.9	nr	3.0		20.7	4.1	2.7	+	6.8
		11	4.0	0.8	28.9	11.5	3.1	7.0	11.4	nr	1.0		20.9	2.2	3.2	+	5.9
	Bay	12	3.1	1.3	24.0	9.1	4.1	6.8	11.9	nr	2.3		20.0	3.1	6.2	+	8.0
	Bay	13	4.3	1.2	29.8	10.9	2.4	7.0	12.6	0.9	1.0		17.7	1.9	4.0	+	7.3
		14	5.0	0.7	25.8	13.4	+	0.5	28.0	+	+		17.5	+	6.9	+	2.3
Crassostrea virginica	Pond	1	5.5	1.1	28.5	5.2	nr	3.5	11.6	3.9	0		9.6	6.7	1.9	5.1	17.3
		5	7.0	1.2	26.1	5.3	2.8	4.6	20.4	2.9	+		10.6	4.7	1.0	5.6	7.8
Mytilus edulis	Pond	15	4.9	3.4	20.8	9.8	7.3	4.5	6.6	--	2.3	--	13.0	6.0	2.4	5.1	14.0
Modiolus demissus	Pond	16	3.9	2.2	33.7	nr	nr	6.8	6.2	--	1.8	--	13.5	8.0	3.3	9.7	11.0

NOTES: [a] more than one component
[b] includes 20:2
nr not resolved
+ trace (less than 1%)

Figure 11. Representative chromatographic distributions of fatty acids in the hard clam, *Mercenaria mercenaria*. A and B from Narragansett Bay, C and D from Pt. Judith Pond, a small estuary located 9 miles southwest of Narragansett Bay.



FATTY ACID METHYL ESTERS

There is also an indication that *Mya arenaria* from Pt. Judith Pond has relatively more 18:4 and less 18:1 than the Bay population.

These preliminary results suggest that nearby populations have environmentally induced differences which do not disappear in 3-1/2 months. A longer observation period will be necessary to determine the genetic and environmental basis of these differences. It seems that the unidentified fatty acid occurring as an unresolved shoulder on the 18:4 peak may represent a genetic difference between Bay and Pond hard clams. A second genetic difference could be the relatively high concentrations of short-chain fatty acids in the pond stock.

Observations were repeated the following summer on the hard clam, but the experiment had to be terminated after six weeks. In this series, analyses were made at collection and after holding the clams in Narragansett Bay to see the effects accompanying a period of rapid change in the food quality and composition.

The clams from upper Pt. Judith Pond had exceptionally low concentrations of 16:0, between 14.6% and 19.4%, but this appeared to be a peculiarity of the year (Table 12). (The concentration was 25.4% in a clam taken here the previous summer, Table 11). After transfer to the Bay the 16:0 content may have decreased in response to changes in the planktonic food (Section III).

The mussels *Mytilus edulis* and *Modiolus demissus* differ qualitatively and quantitatively in fatty acid composition (Table 11). The concentration of 16:0 in *Modiolus* is 33.7% and only 20.8% in *Mytilus*. *Mytilus* has clearly resolved peaks at the 16:1 and 16:2 positions,

comprising 9.8% and 7.3% of the total fatty acids, but in *Modiolus*, these peaks are not resolved, indicating the presence of fatty acids which do not occur in *Mytilus*.

Now that filter feeders can be reliably maintained in the laboratory, this experiment will be conducted over a period sufficient to show the environmental and genetic determinants of fatty acid composition. Amino acid spectra will also be studied.

Table 12. Fatty acid composition of Mercenaria mercenaria from Pt. Judith Pond and Narragansett Bay. The specimens were collected in May, 1965 and held until June (six weeks) in Narragansett Bay. Analyses made immediately after collection (1) and six weeks after (2).

	FATTY ACIDS -- PERCENT OF TOTAL																	
	12:0	14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	20:0	20:1	18:4[b]	22:0	22:1	20:4	20:5	24:1	24:2	22:6
Pt. Judith Pond	+	2.7	+	18.9	10.3	+	6.8	11.3	2.8	+	7.3	3.1	+	5.1	7.4	7.0	4.4	2.4	9.9
Lower Pond	+	3.0	+	24.4	6.2[a]	+	6.2	6.5	2.1	+	7.8	4.2	+	4.6	5.6	12.1	3.5	2.3	11.4
(1) May	+	2.6	+	24.4	9.9[a]	+	7.7	8.1	+ nr	+	9.4	4.3	+	3.8	8.8	7.1	3.6	2.7	7.5
	+	2.0	0.5	22.4	9.7[a]	+	8.1	8.8	1.2	+	9.6	3.1	+	3.2	7.8	8.2	3.2	1.8	10.4
(2) June	+	2.8	+	15.3	9.3[a]	+	9.9	18.8	+ nr	+	5.1	4.8	+	3.8	9.6	9.9	2.1	2.3	6.2
	+	2.2	0.8	21.0	7.8[a]	+	7.1	8.0	1.5	+	8.3	5.9	+	3.9	6.9	10.0	2.9	1.9	11.8
Upper Pond	+	2.8	0.5	17.6	12.5	0.8	6.7	7.2	1.4	+	7.6	3.7	+	4.9	7.7	8.4	4.3	2.9	11.2
(polluted)	0.6	3.9	0.6	19.4	9.7	0.9	5.9	15.4	1.6[a]	+	10.4	2.7	+	5.6	5.3	6.0	3.1	1.6	7.5
(1) May	0.9	4.3	0.7	19.1	9.7	+	8.1	13.0	1.0[a]	+	8.4	3.9	+	4.5	5.4	9.0	2.3	1.3	8.3
	+	1.7	0.8	14.6	7.7	3.3	7.7	7.4	1.0	+	7.6	4.6	+	4.1	10.0	9.9	3.7	3.0	12.8
(2) June	+	4.2	+	18.1	11.9[a]	+	4.5	20.9	1.6	+	11.2	4.2	-	5.9	3.6	6.1	1.8	+	6.0
	1.2	4.1	+	19.4	11.4[a]	+	7.4	6.8	1.5	+	6.3	3.4	0.7	4.2	8.2	9.5	3.1	1.8	11.0
Narragansett Bay	0.9	4.7	0.6	27.8	7.8	+	3.5	8.2[a]	-- 3.3 --		11.7	5.0	-	2.3	4.9	8.9	2.6	1.5	6.1
(1) May	0.4	2.0	0.6	23.2	7.2	1.9	8.1	9.2	-- 1.0 --		8.3	3.3	-	2.5	7.4	9.7	3.7	2.4	8.6
	0.9	4.6	1.3	26.1	6.3	+	7.2	7.5	-- 1.5 --		8.3	4.5	0.6	2.2	7.5	9.6	3.4	1.8	6.7
	0.6	3.3	0.7	24.0	7.1	2.1	6.5	12.0	-- 1.5 --		9.3	2.9	-	3.7[a]	6.9	7.6	2.7	1.9	7.1
	1.8	3.9	0.9	25.1	6.3	+	4.4	11.9	+ nr	+	6.9	4.4	0.9[a]	3.1[a]	6.8	12.1	4.4	1.7	5.3
	0.6	2.8	2.0	22.2	6.5	+	9.2	9.3	3.4	+	4.0	3.5	0.4	3.4	10.5	8.5	4.3	2.2	7.1
	0.4	4.1	1.9	26.6	8.8	+	5.5	10.2	+ nr	+	[a]8.1	3.5	+	2.9	8.6	8.4	3.5	2.1	5.3
	+	4.5	1.8	17.6	4.6	+	5.9	14.4	-- 1.4 --		11.3	5.8	+	3.2	6.6[a]	11.5	3.8	1.8	5.8
(2) June	0.1	1.3	1.7	25.9	6.8[a]	+	9.0	9.1	-- 1.5 --		8.6	3.3[a]	0.3	2.5	8.1	8.5	5.7	3.2	4.5
	+	2.0	0.7	26.2	5.1	+	9.4	9.3	-- 1.9 --		10.2	5.5[a]	-	2.1	9.4	7.1	4.4	3.0	3.4
	+	1.4	0.8	25.1	5.2[a]	+	9.4	10.8	-- 1.6 --		10.8	2.7	+	2.6	9.7	8.3	3.9	2.5	5.3
	+	1.2	2.0	35.4	5.4[a]	+	8.2	9.4	-- 2.0 --		8.2	4.0	+	2.1	6.8	6.7	3.4	2.1	3.5

[a] more than one component

[b] includes 20:2

nr not resolved

\+ trace (less than 1.0%)

VI. References

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RECENT PUBLICATIONS

H. P. Jeffries

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PARTITIONING OF THE ESTUARINE ENVIRONMENT BY TWO SPECIES OF *CANCER*.
Ecology 47:477-481 (1966)

ABSTRACT

There is little mixing of *Cancer irroratus* and *C. borealis* populations in Narragansett Bay, R. I. *Cancer irroratus* is found on sand, and *C. borealis* occurs in areas of coarse gravel and mixed rocky debris. The morphology, walking ability and serum of these crabs were examined to explain their separation in the bay.

Walking ability was measured in a rotating jug. *Cancer irroratus* was at least five times more active and had a higher optimum temperature for walking than *C. borealis*. The optimum temperature for walking corresponded with distributions of both species on the east coast of North America: *C. irroratus* is most common in temperate waters, whereas *C. borealis* is a more northern, colder-water species. *Cancer irroratus* also had high concentrations of serum phosphate, which may be associated with its activity in nature. The concentrations of glucose, total nitrogen and chloride were the same in both species, presumably because their growth rates were similar and the salinity in both habitats was the same. Thus, *C. irroratus* is adapted for life on a flat, sand bottom where survival must depend upon an ability to run, catch food and escape from predators. *C. borealis* leads a reclusive life, hiding in crevices and relying upon its heavy claws for protection.

Spatial partitioning of the bottom by *Cancer* is compared to temporal partitioning in the plankton by two species of *Acartia*. Competition within these groups might help to stabilize estuarine production.

INTERNAL CONDITION OF A DIMINISHING BLUE CRAB POPULATION *(CALLINECTES SAPIDUS)*.
Ches. Sci. 7:164-170 (1966)

ABSTRACT

Abundance of the blue crab, *Callinectes sapidus*, in Rhode Island estuaries has decreased during the last 30 years. A commercial fishery once existed in areas where the blue crab is no longer found.

Composition of the plasma and muscle was studied to see if abnormalities could be recognized. Mean concentrations of total sugars, protein, non-protein nitrogen, phosphate, and chloride did not differ in crabs from two contrasting lagoons; the mean concentrations of lipids and total nitrogen were also independent of the population's habitat. The concentration of sugars was atypically low compared with reports on crabs from North Carolina.

In the plasma, total sugar concentration was directly related to its conductivity; the concentrations of non-protein nitrogen and phosphate were inversely related to chlorinity. Groups of such correlated tests have predictive value and, when further elaborated, they might be applied to the management of the fishery.

SATURATION OF ESTUARINE ZOOPLANKTON BY CONGENERIC ASSOCIATES.
In: Estuaries, edited by G. H. Lauff, p. 500-508. Publication No. 83, AAAS, Wash., D.C. (1967)

SUMMARY

The holoplankton in Atlantic estuaries from Chesapeake Bay to Cape Cod is dominated by a few species capable of developing tremendous populations. The calanoid copepod genera *Acartia* and *Eurytemora* plus the cyclopoid *Oithona* account for the major share, with periodic contributions from the cladocerans *Podon* and *Evadne*. The fauna is simple, consisting primarily of entomostracans. There are, in general, two major species per genus, one species is less affected by low salinity and is more southern in overall geographic distribution than the other. These congeneric associates are also common in coastal waters. Only 3 out of the 23 entomostracans in the estuarine holoplankton are the sole representatives of their respective genera. The 3 exceptions are marine: they do not develop self-sustaining populations throughout an estuary's middle reaches.

The following properties of congeneric associates are examined:
(1) their role in the evolutionary colonization of brackish habitats;
(2) their competitive interactions, which give rise to the high levels of productivity characterizing the rich but demanding estuarine environment;
(3) their ability to partition the environment in time or in space.

The walking ability of congeneric crabs (*Cancer irroratus* and *C. borealis*) determine the type of life each species leads in its own particular habitat. In a device for measuring locomotor endurance, *C. irroratus*, walked for periods up to 18 days, whereas *C. borealis*, ran less than 20% as well. In nature, *C. irroratus* inhabits an exposed sand surface where high mobility is necessary to escape from predators and obtain food; the reclusive *C. borealis* emerges from natural concealment on a stony bottom to capture food and ward off predators with its heavy powerful claws. Serum phosphate concentrations in natural populations differed between species.

This corresponded to the levels of muscular metabolism required in each habitat, but serum nitrogen and glucose concentrations were the same for each member of the pair since osmoregulatory burdens were the same for both species.

Spatial separation of benthic crabs is compared with the temporal succession of planktonic congeners *(Acartia tonsa* and *A. clausi)*. Both methods of partitioning the biotope (spatial segregation on the bottom and temporal segregation in the water) contribute to the derivation of maximum power output in a constantly changing environment.

SEASONAL CYCLES OF FATTY ACIDS AND AMINO ACIDS IN TEMPERATE ZOOPLANKTON COMMUNITIES.

Second International Oceanographic Congress. Abstracts of Papers. Publishing House, Nauka, Moscow. p. 179-180. (1966)

ABSTRACT

Seasonal succession of plankton populations in Temperate waters results from a series of stresses that are imposed by the annual cycle of temperature variation. To indicate the severity of thermal stress prior to species succession, fatty acids and amino acids were extracted from net plankton samples and analyzed chromatographically. Patterns of species succession were compared quantitatively with the various organic acids during an annual cycle in Rhode Island Sound and Narragansett Bay, Rhode Island.

During the spring diatom bloom, palmitoleic acid in the phytoplankton increased from 17% to 35% of the total fatty acids. This occurred first in the Sound (April) and then in the Bay (May). The phytoplankton then changed from a *Skeletonema costatum* diatom community to a community dominated by several flagellate species (June). The increase in palmitoleic acid preceeded the change in dominance from diatom to flagellate community, which also took place first in the Sound and then in the Bay. Palmitoleic acid dropped abruptly to 5-8% during succession, and palmitic acid increased almost four-fold, from 11% to 40%. In addition, the pigment ratio D_{430}/D_{665} was low at the end of the spring diatom bloom, indicating that an increase in biotic diversity would soon take place. Thus, species succession in phytoplankton was intimately associated with successive changes in the chemical composition of the community.

The relative concentration of each fatty acid varied less in the zooplankton than in the phytoplankton, and the levels were less variable over time in the Sound than in the Bay. During February, increases in palmitoleic and oleic acids accompanied the dispersal of *Sagitta elegans* from offshore. This adventitious species did not propagate in the Bay, yet it caused a major shift in the community's chemical composition. In late June and July, the fatty acid pattern of the zooplankton changed again. Myristic and palmitoleic acids increased 20% in the Bay community, while palmitic and eicosapentaenoic acids decreased lesser amounts. Seasonal succession took place at this time: the winter-spring copepods *Acartia clausi, Pseudocalanus minutus* and *Centropages hamatus* were replaced by the more thermophilic

species *Acartia tonsa*, *Temora longicornis* and *Centropages typicus*, which propagate during the summer and fall. When the water cooled again in the fall, the pattern of relative concentrations returned to the winter condition.

Amino acid concentrations also changed when the summer species replaced the winter species, but the variations were small compared to the shifts in fatty acids at this time. As was the case for the fatty acids, amino levels were more stable in the Sound than in the Bay. Distribution and abundance of estuarine species vary tremendously. Thus, it appears that the organic acids mirror not only seasonal succession, but they also indicate community stability in different habitats.

Future studies will show the specific responses of homeostatic systems to natural and artificial stress. Injury or unfavourable conditions may evoke stereotyped responses within the individual organism and perhaps the community as well. If so, we should be able to determine the well-being of commercial species and clarify their relation to the supporting community. Since pollution is now a threat to coastal populations, trends toward the abnormal occurring within the "normal" population should be recognized before the damage is permanent and valuable fisheries are injured.

STUDENT INVESTIGATIONS

The Plasma Protein Variations of Three Estuarine Fish Species

Gerald G. Pesch

ABSTRACT

Variations in serum protein composition of three fish species were compared: *Pseudopleuronectes americanus*, (winter flounder), *Urophycis tenuis*, (white hake), and *Merluccius bilinearis* (silver hake). The dominant fractions in each species were the alpha and beta globulins (Fig. 1). An albumin fraction had greater mobility than human albumin. Species-specific patterns were superimposed on a wide range of intraspecific variation. The extent of this variation was statistically estimated on large numbers of individuals.

The variations in a *Pseudopleuronectes americanus* population were associated with age, sex, sexual maturation and disease. Total protein concentration increased with age. Sexual maturation was accompanied by marked increases in protein concentration: the slow fraction increased in females, and the fast fraction increased in males (Fig. 2). Diseased fish had low protein concentrations (Fig. 3).

The variations of serum proteins occurring in estuarine fish populations are discussed in relation to the physical nature of the habitat.

Figure 1. Densitometry records of serum protein distributions in *Merluccius bilinearis* (silver hake), *Urophycis tenuis* (white hake) and *Pseudopleuronectes americanus* (winter flounder). Electrophoresis on cellulose acetate strips stained with Ponceau S.

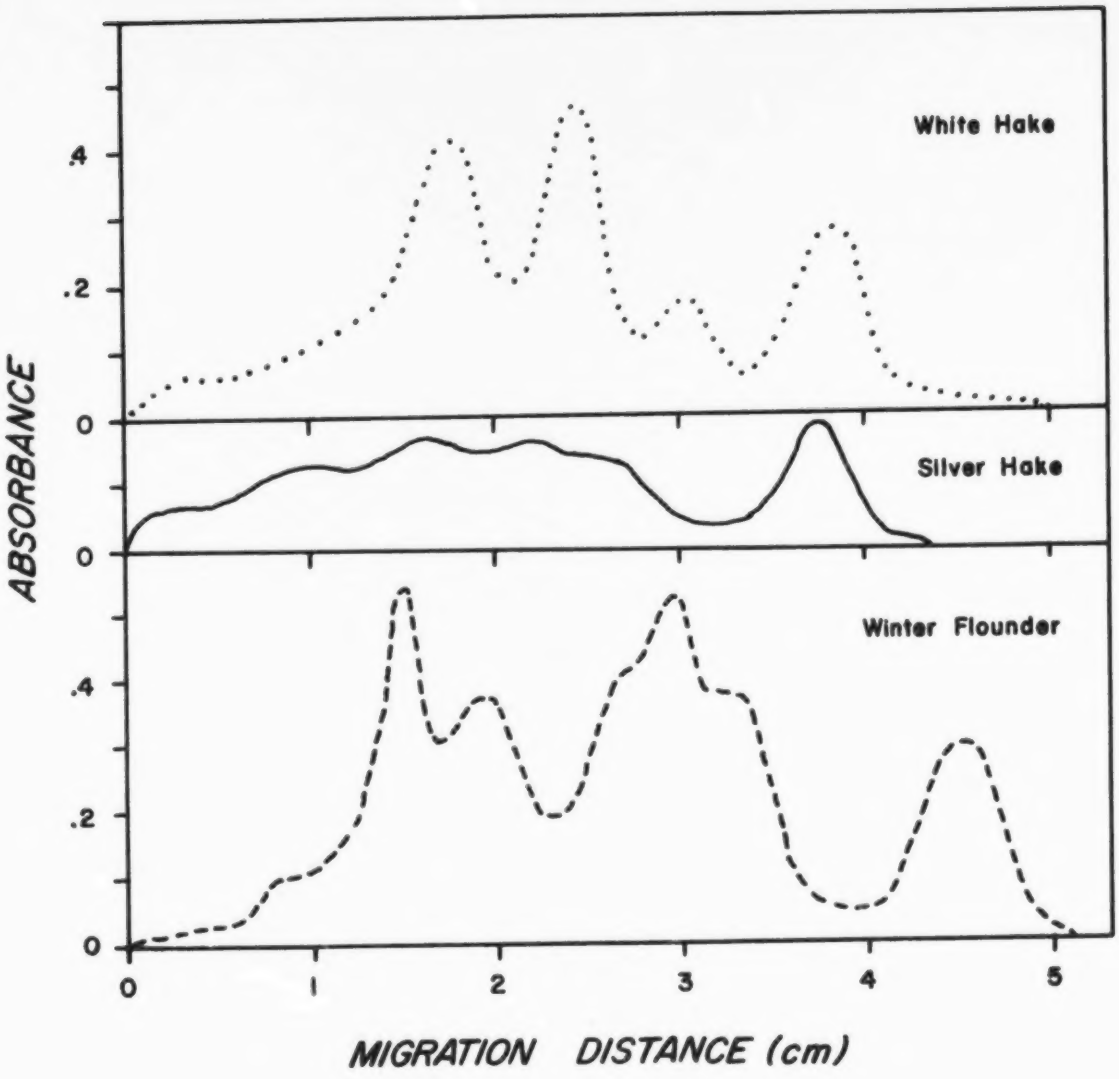

White Hake
Silver Hake
Winter Flounder
ABSORBANCE
.4
.2
0
0
.4
.2
0
0
1
2
3
4
5
MIGRATION DISTANCE (cm)

Figure 2. Densitometry records of serum protein distributions in mature and immature individuals of *Pseudopleuronectes americanus*.

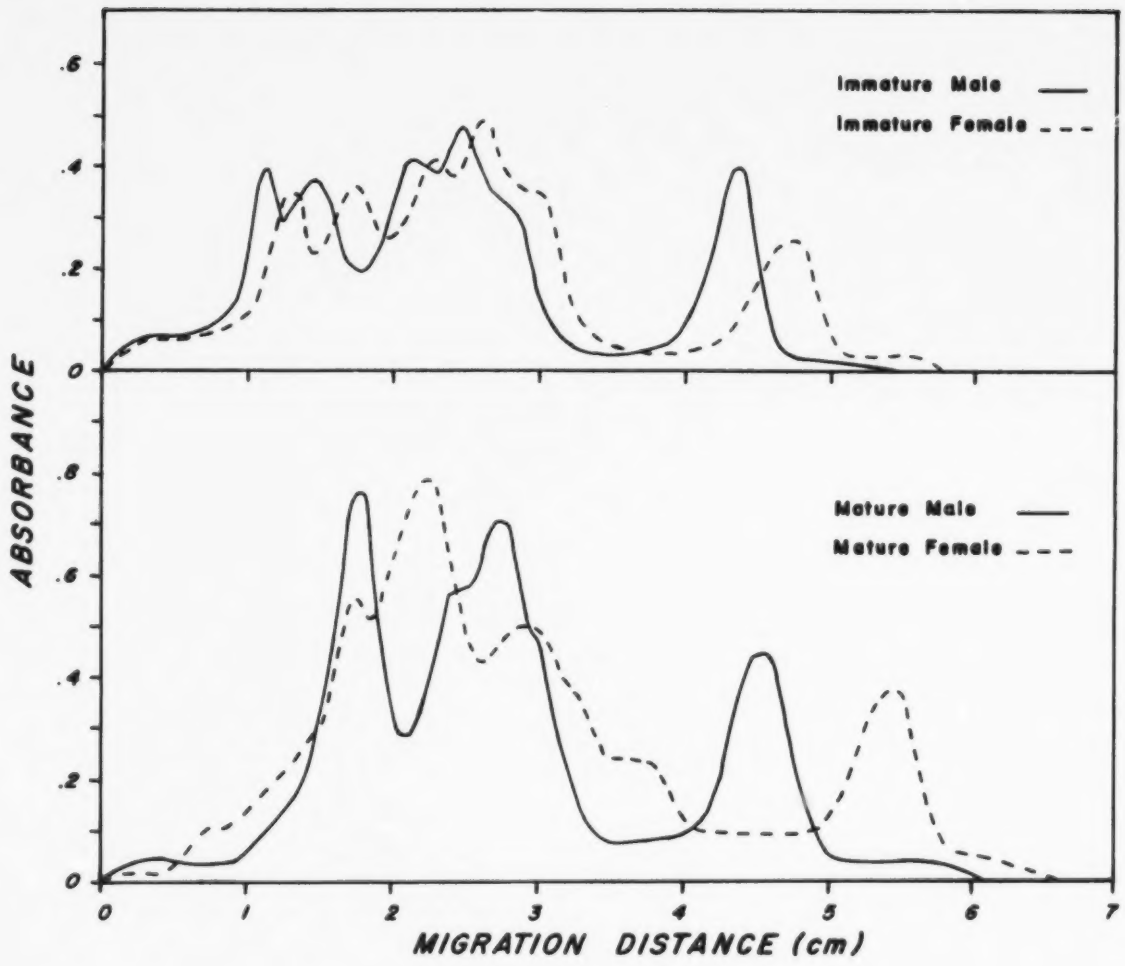
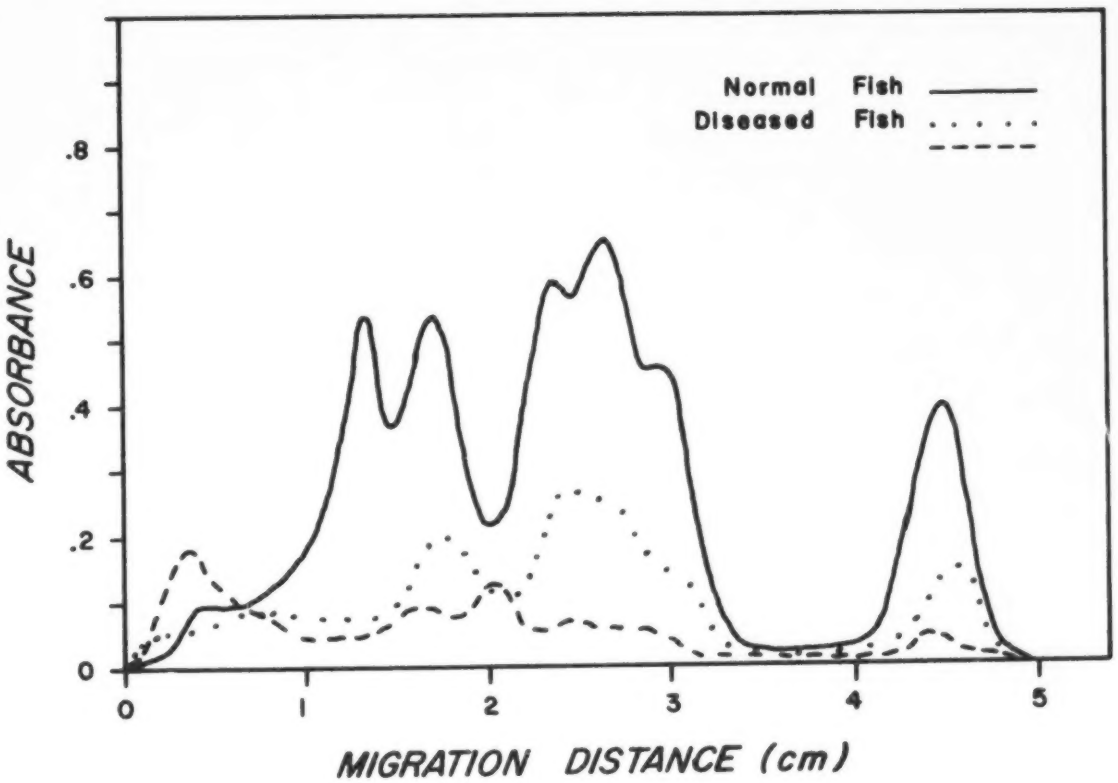

Immature Male
Immature Female
Mature Male
Mature Female
ABSORBANCE
MIGRATION DISTANCE (cm)
0
.2
.4
.6
1
2
3
4
5
6
7

Figure 3. Densitometry records of serum protein distributions in normal and diseased individuals of *Pseudopleuronectes americanus*.


Normal Fish
Diseased Fish
ABSORBANCE
.8
.6
.4
.2
0
0
1
2
3
4
5
MIGRATION DISTANCE (cm)

The Ecological Significance of the Free Amino Acid Pool in *Gemma gemma*

J. Ross Wilcox

ABSTRACT

In the small pelecypod, *Gemma gemma*, the free amino acid pool responds to environmental change. Salinity, food, and sediment conditions are associated with the levels of the 19 free amino acids that comprise the pool in *Gemma*.

In 50% sea water (15 °/oo), the free amino acids compensate for the drop in osmotic concentration of the external environment. Taurine, a metabolic by-product, decreases more than the other free amino acids, and thereby has a sparing effect on the essential amino acids.

In 20% sea water, feeding stops and food reserves are used at a rate that is proportional to temperature. Exhaustion of lipids and carbohydrates is accompanied by proteolysis. Large amounts of free amino acids are released for metabolism. Upon exhaustion of the amino acids, death occurs.

The response of free amino acids to a change in sediment show the classic pattern of over- and under-compensation followed by acclimation to a new steady state.

The ecological significance of the responses is discussed.

A Survey of the Distribution of Free Amino Acids in Five Species of Marine Copepods

Laima Alzara

PROGRESS

A survey was made of the free amino acids in natural populations of five copepod species that occur along a salinity gradient extending from Pettaquamscutt River to Narragansett Bay and Rhode Island Sound. (Table 1).

The following species were studied:

Species	Classification (Jeffries 1967)	Optimum Salinity Range o/oo
EURYTEMORA AFFINIS	True estuarine	5-15
ACARTIA TONSA	Estuarine & marine	15-30
ACARTIA CLAUSI	Estuarine & marine	15-31
PSEUDOCALANUS MINUTUS	Euryhaline marine	27-34
CALANUS FINMARCHICUS	Stenohaline marine	30-35

Eurytemora is a TRUE ESTUARINE form in the sense that it can propagate only in brackish water. (Jeffries 1967). Both *Acartia* species are dominant members of the zooplankton in New England estuaries. They represent the ESTUARINE & MARINE group: both propagate at salinities down to 10 °/oo, but *A. tonsa* adapts better at the lower end of the range. *Calanus finmarchicus* is an open ocean form that drifts into brackish water in winter and spring.

Two questions were asked: (1) do copepod species have characteristically different patterns of free amino acids and, (2) is there a relationship between the salinity range of the species and its free amino acid pool?

TABLE I

Environmental data, % abundance and total free amino acids of copepod species from Pettaquamscutt River, Narragansett Bay, and Rhode Island Sound. Total free amino acids are expressed as µMoles/gram dry weight. Pett. R. (Pettaquamscutt River), Nar. B. (Narragansett Bay), RIS (Rhode Island Sound). December, 1965 - March, 1967.

Species	Month	Location	Salinity °/oo	Temp °C	Abundance %	Total Free Amino Acids, µM/g dry wt.
Eurytemora affinis	Feb	Pett. R.	5-15	1.0	90.0	217.5
	Mar		5-15	2.0	98.0	201.8
Acartia tonsa	Aug	Nar. B.	31.83	20.6	99.6	340.9
	Oct		31.85	15.5	99.7	399.7
	Oct		31.69	15.5	96.2	352.1
	Oct		31.80	14.0	98.1	549.2
Acartia clausi	Dec	Nar. B.	30-32	6.4	90.2	659.9
	Dec		30-32	3.0	81.5	614.7
	Apr		30-32	6.3	82.!	595.1
Pseudocalanus minutus	Dec	RIS	32-33	6.3	89.0	614.7
	Jan		32-33	---	82.8	642.5
Calanus finmarchicus[a]	May	Plymouth England	---	---	100.0	487.0

[a]Cowey and Corner (1963)

The relationship between total free amino acids and the corresponding optimum salinity of each species is curvilinear (Table 2). *Acartia clausi* and *Pseudocalanus minutus*, species subject to the greatest environmental fluctuations, have the highest total concentrations. The more euryhaline species, *Eurytemora affinis* and *Acartia tonsa*, have lower levels than *Calanus finmarchicus*, an oceanic species.

Free amino acid distributions within the pool differ among the five species (Table 2). The variations are especially striking with respect to glycine, proline, taurine, alanine and arginine. With the exception of arginine, these amino acids may be involved in osmoregulation (Florkin and Schoffeniels 1965).

In *Eurytemora*, the most abundant amino acid is alanine, but glycine predominates in the other four species. *Eurytemora* is also distinguished by an absence of histidine.

Acartia tonsa has lower concentrations of glycine, proline, taurine and alanine than *Acartia clausi*. The two species have similar concentrations of aspartic acid, serine, methionine and lysine. The lower levels in *Acartia tonsa* may indicate compensatory responses to the estuarine environment. This supports, in part, the speculations of Lance (1963) and Jeffries (1962) that *A. tonsa* has a more efficient osmoregulatory system than *A. clausi*.

TABLE 2

Mean free amino acid concentration (µMoles/gram dry weight) in the extracts of copepod species from Pettaquamscutt River, Narragansett Bay and Rhode Island Sound. N is the number of samples analyzed. 1965 - 1967.

Amino Acid	*Eurytemora affinis*	*Acartia tonsa*	*Acartia clausi*	*Pseudocalanus minutus*	*Calanus finmarchicus*[a]
CYS	2.0	1.9	3.3	2.3	---
TAU	29.3	42.1	86.4	78.5	85.7
ASP	2.2	5.1	5.7	4.9	4.0
THR	5.8	11.1	13.6	12.1	9.5
SER	6.9	10.4	10.7	8.3	13.8
GLU	12.8	12.4	17.0	24.5	14.3
PRO	27.8	72.4	98.6	115.8	41.5
GLY	19.9	101.2	196.0	170.9	119.7
ALA	41.1	44.8	65.8	70.6	41.5
VAL	5.3	12.1	14.5	15.4	13.6
MET	3.0	5.1	5.5	5.6	6.3
ILEU	3.9	7.4	9.4	11.1	11.6
LEU	6.3	13.6	15.8	17.9	16.6
TYR	3.3	5.2	7.1	10.1	10.6
PHE	3.9	5.7	8.0	10.3	7.5
LYS	8.6	18.1	18.4	15.9	17.4
HIS	---	3.4	4.3	5.9	4.9
ARG	26.1	38.0	55.3	48.6	68.7
TOTAL	209.7	410.4	635.3	628.6	487.0
N	2	4	3	2	1

[a]Cowey and Corner (1963)

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Lance, J. 1963. The salinity tolerance of some estuarine planktonic copepods. Limnol. Oceanogr. 8:440-449.

S T A F F I N G

Name	University Title	Period	Time
H. Perry Jeffries	Associate Professor of Oceanography	10/1/65-present	80%
Heidimarie Myers	Research Assistant	9/14/66-present	100%
Laima Alzara	Graduate Assistant	9/1/66-present	50%
Gerald Pesch	Graduate Assistant	10/1/65-present	research
J. Ross Wilcox	NDEA Fellow	6/1/66-present	research
Evelyn Dyer	Laboratory Assistant	10/1/65-present	12 hrs/wk
Lucy Chang	Laboratory Assistant	10/1/65-9/30/66	8 hrs/wk
Paola Mangiacapra	Research Assistant	10/1/65-8/30/66	100%
Betty Ann Whitney	Laboratory Assistant	10/1/65-9/30/66	8 hrs/wk
Barbara McDaniel	Laboratory Assistant	10/1/65-10/7/65	16 hrs total